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ENVIRONMENTAL ANALYSIS

DOUGLAS-FIR TUSSOCK MOTH MICROBIAL INSECTICIDE PILOT TESTS

1974

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USDA FOREST SERVICE ENVIRONMENTAL ANALYSIS
DOUGLAS-FIR TUSSOCK MOTH
PILOT TEST PLAN
NORTHERN IDAHO - 1974

CONTENTS

I. DESCRIPTION 1
Proposed Action 1
Douglas-fir Tussock Moth Biology 1
Impact of Defoliation on Trees 2
Brief History of Past Epidemics and Suppression Activities 4
Appraisal of Impacts 4
Entomological Evaluation Summary 11
II. ENVIRONMENTAL IMPACTS 12
Safety Evaluation 16
III. FAVORABLE ENVIRONMENTAL EFFECTS 19
IV. ADVERSE ENVIRONMENTAL EFFECTS 20
V. ALTERNATIVES TO THE PROPOSED ACTION 21
No Action (normal timber sale-salvage activity) 21
Accelerated Salvage 21
Direct control using chemicals 23
Zectran 23
Stabilized pyrethrins 23
Carbaryl (Sevin). 24
Trichlorfon (Dylox) 24
DDT 24
VI. RELATIONSHIP BETWEEN LOCAL SHORT-TERM USES OF MAN'S ENVIRONMENT AND THE MAINTENANCE AND ENHANCEMENT OF LONG-TERM PRODUCTIVITY 26
VII. IRREVERSIBLE OR IRRETRIEVABLE COMMITMENT OF RESOURCES 27
VIII. SUPPORTING RESEARCH AND DEVELOPMENT PROGRAMS 28
Development of Microbial Insecticides 29
Development of Chemical Insecticides 30
IX. REFERENCES CITED 31
X. APPENDIX 32
Section A: Work plan for pilot test . . . . . .
Section B: History of past epidemics and suppression activities . . . . . .
Section C: Entomological evaluation . . . . . .
Section D: Background data on *Bacillus thuringiensis* . . . . . .
Section E: Background data on the nuclear polyhedrosis virus . . . . . .

I. DESCRIPTION

The USDA Forest Service proposes to pilot test two biological control agents, *Bacillus thuringiensis* and a nuclear polyhedrosis virus, on the Douglas-fir tussock moth (*Orgyia pseudotsugata* McDonnough) outbreak in northern Idaho. The purpose of the proposed action is to obtain more data on both *B. thuringiensis* and virus, hopefully leading to registration of one or both for control of the tussock moth. Background data on *B. thuringiensis* is given in Appendix D. Background data on the nuclear polyhedrosis virus is given in Appendix E.

Biotic conditions favorable for explosive tussock moth outbreaks are unknown. The history of tussock moth outbreaks indicates populations can reach high levels during a 3- to 4-year period and cause heavy mortality in Douglas-fir and true fir stands. Populations within the outbreak area of 99,000 acres may increase until weather, food supply, a naturally occurring virus, and other biotic factors cause a population collapse. Historically, these factors are not effective until the end of the third year after the outbreak develops and many trees have been killed.

Proposed Action

This analysis indicates that DDT is the only proven insecticide for the control of tussock moth. Pilot testing of these two microbial insecticides, *B. thuringiensis* and the polyhedrosis virus, is needed to develop more environmentally acceptable control measures. Such a pilot test is proposed on 17,500 acres. A copy of the work plan for the pilot tests is attached as Appendix A.

The total number of acres that will be affected is contingent upon the incidence of the polyhedrosis virus in the population and climatic factors which may cause the outbreak to collapse. Biological evaluations will continue into early egg hatch in June 1974. Results of these evaluations may modify the pilot testing proposed in this statement.

Douglas-fir Tussock Moth Biology
(based on Anonymous, 1969; Wickman et al, 1971)

Tussock moths get their name from the four dense, buff-colored tussocks of hairs along the middle of the back of mature larvae. Larvae are about 1¼ inches long when fully grown and have two long, dark tufts or pencils of hairs back of the head; a similar but longer pencil of hair is on the posterior end. Shorter hairs cover the body and grow out of red, button-like centers. Body hairs are barbed and cause a skin rash on some people.

The female moth is about three-fourths of an inch long, grayish in color, has short thread-like antennae, and tiny undeveloped wings which cannot be used for flying. She is almost all abdomen.

Males have well-developed wings for flying, are grayish in color, and have feathered antennae. Their wingspread is 1 to 1¼ inches.

Tussock moths overwinter in the egg stage. Eggs hatch from mid-May to early June. Newly hatched larvae are one-eighth inch long, gray, and have long hairs. This combination of light weight and long hair allows the larvae to be transported long distances by the wind.

Larvae in the first three stages of development feed on current year's needles. Larvae in the last two or three stages feed on older needles, sometimes until the end of July. Mature larvae make a thin cocoon of silken webbing mixed with body hairs and go into the pupa, or resting stage.

Pupae are often found in large masses on the foliage or trunks of host trees, on ground plants, under rocks, boards, and eaves of buildings near infested trees. Moths emerge from cocoons 10 to 18 days after pupation. The female moth cannot fly; she clings to the outside of the cocoon and the male moth is attracted to her. After mating, females lay eggs on the surface of the cocoon in a mass of frothy, gelatinous substance containing many body hairs. As many as 250 white eggs are laid by each female in three layers within a mass.

The tussock moth has many natural enemies, including disease organisms, insect parasites and predators, and birds. Probably most important is a nucleopolyhedrosis virus disease that is capable of wiping out high populations, but it usually appears only after trees have been seriously defoliated. This disease kills both larvae and pupae and is usually present in some degree within all populations.

Impact of Defoliation on Trees

The tussock moth prefers to feed on Douglas-fir, white fir, and grand fir. It will occasionally feed quite heavily on subalpine fir. Larvae have been found feeding on ponderosa pine, western hemlock, and western larch after the preferred hosts have been defoliated, but these species usually are not severely damaged. All feeding is done during the larval stage. The young larvae eat the underside of new needles. Later, older needles are eaten, or severed near the base and left entwined in silken webbing. Defoliated trees turn reddish brown by July. Lower elevation stands are affected first. Tree tops are usually defoliated first and may be heavily webbed. Infested trees can be completely stripped in one season and may die or produce a dead top. Studies of California outbreaks in white fir stands occurring in 1935-37, and 1954-56 (Wickman, 1958 and 1963) and in 1963-65 (Wert and Wickman, 1970) have shown that immediate damage consists of tree mortality, top kill, and radial growth reduction.

The California studies (Wickman, 1958 and 1963; Wert and Wickman, 1970) show that tree mortality can be very severe when white fir stands are 75 to 100 percent defoliated. This mortality does not occur uniformly

throughout the stand. Scattered groups of trees, 1 to 50 acres in size (sometimes larger), have higher tussock moth populations than others; most of the significant damage occurs in these clumps. These clumps usually represent about 14 percent of the total infestation. Tree mortality in these clumps may be as high as 94 percent; average mortality is 84 percent. Highest losses occur in old-growth fir stands.

The degree of defoliation influences tree mortality. Trees with less than 50 percent defoliation have a higher survival rate than trees more severely defoliated; and survival is higher in second-growth stands than in old-growth stands (Wickman, 1963).

Where defoliation is not severe enough to cause tree mortality, the upper part of the crown often dies. About half of the trees that are 50 to 75 percent defoliated exhibit top kill. This damage is more prevalent in merchantable second-growth stands (26 percent) than in old-growth stands (12 percent).

The tussock moth often kills the terminal shoot on small trees, but damage is usually not severe. Most of the damaged trees develop new leaders from lateral branches.

Defect due to decay organisms in top-killed white fir in a California tussock moth outbreak was not serious 33 years after defoliation (Wickman and Scharpf, 1972).

Tussock moth defoliation also affects radial tree growth. The annual ring is normally wider in the upper part of the stem than at the base. Immediately after an outbreak, the growth pattern changes. Defoliation causes significant decreases in ring width at all levels, but the magnitude of the decrease is proportionally greater in the upper part of the tree (Wickman, 1963). In a California outbreak area, Wickman (1963) found the average growth reduction during the 3 years of growth during the four years preceding defoliation, was as follows: heavy defoliation, 74 percent; moderate defoliation, 67 percent; light defoliation, 31 percent. Growth recovery was not complete until the fourth or fifth year. Translating this into board feet for merchantable trees on one heavily defoliated area, Wickman (1963) showed a growth reduction of 63.5 percent for the 3-year period.

Long-term effects on heavily defoliated white fir areas in California showed an increase in growth on individual trees (Wickman and Scharpf, 1972). If stands are understocked due to tree mortality, this growth increase for survivors may not equal the total expected growth per acre. If surviving trees represent an adequate stocking level, however, salvage plus the long-term growth increase could offset the original tree mortality.

Bark beetle outbreaks sometimes develop in fir stands weakened by tussock moth defoliation. Attacks by the fir engraver, *Scolytus ventralis* Lec., and the roundheaded borer, *Tetropium abietis* Fall, were the second most important cause of tree mortality in California outbreaks (Wickman, 1958). In one infestation, 75 percent of the total tree mortality was caused by beetles for 4 years following defoliation (Wickman, 1958).

Similar impact studies on tussock moth defoliation in grand fir and Douglas-fir stands in Oregon, Washington, and Idaho were established in 1972. Results of these studies will not be available for several years, but are expected to correspond with the white fir data in California.

Brief History of Past Epidemics and Suppression Activities

(Abstract of Tunnock, 1973. See Appendix B.)

The Douglas-fir tussock moth, *Orgyia pseudotsugata* McD., periodically defoliates Douglas-fir, true firs, and other host trees in forests of the western United States. In the Northern Region, these infestations occur about once every decade.

This history covers the earliest recorded outbreak in northeastern Washington from 1928 to 1930 and includes information about outbreaks in northern Idaho and eastern Washington from 1944 to 1974, northeastern Washington and northern Idaho from 1950 to 1955, northern Idaho and western Montana from 1961 to 1965, and the current outbreak in northern Idaho, northeastern Washington, and western Montana which began in 1970.

These outbreaks usually last from 2 to 4 years in forested areas and typically go through a buildup phase the first year, an outbreak phase the second year, and a declining phase the third year due to parasites and/or polyhedrosis virus. Exceptions are: It may take an outbreak 2 years to build up or occasionally natural control agents can cause a population collapse at the end of the second year.

Appraisal of Impacts

Aerial surveys conducted in 1973 by the USDA Forest Service and the Idaho Department of Public Lands detected defoliation caused by the tussock moth on approximately 99,000 acres of Douglas-fir and true fir type in northern Idaho (Table 1). Significant tree mortality did not occur.

Table 1.--Acres of defoliation by ownership and damage class in 1973

Areas	Acres dead	Acres of defoliation by damage class 1/			
		Class I	Class II	Class III	Total
Nezperce Unit					
Nezperce NF 2/	0	1,000	0	22,000	23,000
Craigmont Unit					
State & Private	0	500	1,000	2,500	4,000
Clearwater Unit					
State & Private	0	0	120	0	120
St. Joe Unit					
St. Joe NF	0	1,000	3,000	16,000	20,000
Coeur d'Alene IR	0	300	1,700	0	2,000
State & Private	0	3,700	1,800	42,500	48,000
Subtotal	0	5,000	6,500	58,500	70,000
Coeur d'Alene Unit					
Coeur d'Alene NF 2/	0	200	800	800	1,800
State & Private	0	0	30	50	80
Subtotal	0	200	830	850	1,880
TOTAL	0	6,700	8,450	83,850	99,000

1/ Damage Class

I Fifty percent or more of the host type has been completely defoliated.

II Fifty percent or more of the host type has at least the top quarter of the crown completely defoliated.

III Host type has defoliation visible from survey aircraft. The current year's foliage has been removed on most trees but less than a quarter of the crown has been completely defoliated.

2/ Areas to be used in pilot tests.

Data collected during insect egg surveys in the fall of 1973 indicated that significant tussock moth populations were distributed over slightly more than 223,000 acres. On the basis of egg mass density and new-to-old egg ratio data, and the exclusion of nonhost type, it is predicted that tussock moth populations will cause defoliation on nearly 104,000 acres (Table 2). A continuation of the infestation threatens to cause a serious disruption of timber productivity, an impact on big game habitat, land development values, and recreation esthetic values which may in some instances be far greater than timber values, increase the potential for health hazard, and greatly increase fire hazard in heavily defoliated stands.

The implications of the outbreak in terms of present and projected timber losses and other resource impairments have been summarized from impact data developed in the "USDA-USDI Environmental Impact Statement, Cooperative Douglas-fir Tussock Moth Pest Management Plan, Idaho-Oregon-Washington," filed with Council on Environmental Quality in December 1973.

Timber.--Natural regeneration does not occur on the Selway District, Nezperce National Forest; all regeneration is artificial. The District has about 5,400 acres of host type plantations in old clearcuts within the infested area. These plantations range from 1 to 12 years of age, and probably will suffer considerable mortality.

Lack of a seed crop, due to the western budworm (*Choristoneura occidentalis* Freeman), will result in insufficient seed for nursery grown trees for artificial regeneration of the infested area on the Selway District.

A summary of timber losses due to the 1973 infestation is given in Table 3, and a summary of predicted timber losses due to the 1974 infestation is given in Table 4.

Fire.--Although the fire hazard will increase, specific presuppression plans cannot be made until the pattern of mortality is known. No manpower or money requirements can be determined at this time.

Wildlife.--The impact of tree mortality on wildlife habitat varies with size, density, location, and age of forest stands destroyed and the subsequent management activities by landowners. Impact of the tussock moth may be beneficial. The vegetative changes resulting from tree mortality will create less favorable habitat for some species while improving conditions for others. Most herbivores, predators upon the herbivores, and birds will prosper from the change.

Any increase in forage on the Nezperce and Coeur d'Alene Units will be available for big game.

Watershed.--The impact of tussock moth infestations on watershed quantity and quality should remain minimal if care is taken during the pilot testing or when harvesting damaged timber. Harvesting damaged timber could cause the greatest adverse impact.

Table 2.--Cumulative Acres: Projected (1974) areas of tussock moth defoliation damage by ownership and damage classes if no direct control action is taken.

Area	Total acres dead	Acres of defoliation by damage class				
		Class I	Class II	Class III	Class IV	Total
Criagmont Unit						
State and private	70	446	1,750	2,472	24	4,762
St. Joe Unit						
St. Joe NF	714	1,670	7,072	5,146	245	14,847
Coeur d'Alene I.R.	318	114	328	204	24	988
State and private	2,119	3,292	13,483	10,855	519	30,268
Subtotal	3,151	5,076	20,883	16,205	788	46,103
Coeur d'Alene Unit						
Coeur d'Alene NF 1/	282	1,575	5,846	10,139	27	17,869
State and private	10	88	331	568	1	998
Subtotal	292	1,663	6,177	10,707	28	18,867
Clearwater Unit						
Clearwater NF	7	147	541	1,052	0	1,747
State and private	24	235	868	1,558	1	2,686
Subtotal	31	382	1,409	2,610	1	4,433
Nezperce Unit						
Nezperce NF 1/	672	2,778	11,328	13,338	285	28,401
State and private	190	121	366	626	9	1,312
Subtotal	862	2,899	11,694	13,964	294	29,713
TOTAL	4,406	10,466	41,913	45,958	1,135	103,878

1/ Areas to be used in pilot tests.

Table 3.--Summary of timber losses by ownership resulting from observed defoliation in 1973.

Ownership	Acres	Mortality volume MMBF	Growth loss volume MMBF	Salvage volume MMBF	Value mortality	Pnw 1/ growth loss	Salvage value	Reforest-ation expenses	Net loss
					Millions of dollars				
Criagmont Unit									
State and private	4,000	4.1	0.7	2.1	0.185	0.018	0.062		0.141
St. Joe Unit									
St. Joe NF	20,000	5.7	2.6	4.5	.193	.011	.005		.199
Coeur d'Alene I.F.	2,000	7.0	.8	6.3	.473	.021	.225		.269
State and private	48,000	25.3	6.7	12.7	1.139	.184	.380		.943
Subtotal	70,000	38.0	10.1	23.8	1.805	.216	.610		1.411
Coeur d'Alene Unit									
Coeur d'Alene NF 2/	1,800	1.2	.3	1.0	.042	.001	.001		.042
State and private	80	.1	.1	.1	.001	.001	.001		.001
Subtotal	1,880	1.3	.4	1.1	.043	.002	.002		.043
Clearwater Unit									
State and private	120	.1	.1	.1	.005	.001	.002		.004
Nezperce Unit									
Nezperce NF 2/	23,000	4.1	2.7	1.6	.138	.011	.002		.147
TOTAL	99,000	47.6	14.0	28.7	2.176	.248	.678		1.746

1/ Present net worth.

2/ Areas to be used for pilot tests.

Table 4.--Summary of projected (1974) timber losses by ownership resulting from new or continued defoliation.

Ownership	Acres	Mortality volume MMBF	Growth loss volume MMBF	Salvage volume MMBF	Value mortality	Pnw 1/ Growth loss	Salvage value	Reforest-ation expenses	Net loss
					- - - - - - - Millions of dollars - - - - - - -				
Craigmont Unit									
State and private	4,762	6.0	0.4	3.0	0.268	0.011	0.089	0.017	0.207
St. Joe Unit									
St. Joe NF	14,927	13.3	.9	10.7	.454	.006	.011		.449
Coeur d'Alene I.R.	4,500	9.9	.8	9.4	.672	.020	.319	.036	.409
State and private	45,352	62.4	4.1	31.2	2.809	.118	.936	.270	2.261
Subtotal	64,779	85.6	5.8	51.3	3.935	.144	1.266	.306	3.119
Coeur d'Alene Unit									
Coeur d'Alene NF 2/	29,966	16.5	2.9	13.2	.560	.009	.013		.556
State and private	4,172	3.6	.3	1.8	.162	.007	.054	.016	.131
Subtotal	34,138	20.1	3.2	15.0	.722	.016	.067	.016	.687
Clearwater Unit									
Clearwater NF	2,686	.9	.3	.7	.031	.001	.001		.031
State and private	1,747	2.2	.4	1.1	.101	.012	.034	.010	.089
Subtotal	4,433	3.1	.7	1.8	.132	.013	.035	.010	.120
Nezperce Unit									
Nezperce NF 2/	32,189	25.6	3.1	10.2	.871	.018	.010		.879
State and private	1,312	1.1	.2	.6	.050	.006	.017	.005	.044
Subtotal	33,501	26.7	3.3	10.8	.921	.024	.027	.005	.923
TOTAL	141,613	141.5	13.4	81.9	5.978	.208	1.484	.354	.056

1/ Present net worth.
2/ Areas to be used for pilot tests.

On the Selway District, Nezperce NF, cutting at the infestation elevation is now very near the hydrologic limit. This entire area drains into either the Selway River or the Middle Fork Clearwater River. Two-thirds of the area drains into Clear Creek. This creek is the water source for the Kooskia National Fish Hatchery; any salvage in this area would have to be conducted with extreme care in order not to damage the water quality for the hatchery.

The watershed on the Coeur d'Alene Unit is in good condition. Trees are in the intermediate age class, and very little clearcutting has occurred. The soil is stable, and any new roads required for salvage would cause a minimum of damage if care is taken in layout and construction. Some of the area is already roaded. There would be an increase in water flow and a change in timing of peak flow. The entire area drains into either the Coeur d'Alene River or Lake Coeur d'Alene, and the increase in runoff could be absorbed with no impact.

Recreation.--Recreation impact on the Selway District will be mostly visual. Part of the area is visible from the Selway River, which is classified as a Wild and Scenic River. The Selway is heavily used by recreationists.

The infested area on the Coeur d'Alene Unit is in the viewing area of Interstate 90, Beauty Creek campground, the Coeur d'Alene River, Lake Coeur d'Alene, U.S. 95 south of Coeur d'Alene, and the city of Coeur d'Alene. The Mount Coeur d'Alene picnic area is within the infested area. Most of the impact will be visual, with the exception of the picnic area which will suffer a loss in visitor use because of the hazard to health caused by tussock moth hairs.

Range.--The Selway District grazing allotment carries 170 cattle from June through October. The range is marginal on the Coeur d'Alene Unit.

Any increase in forage on the infested areas would not increase the present numbers of livestock, but would be available for big game.

Primary plants that colonize openings on the Coeur d'Alene Unit are ninebark (*Physocarpus malvaceus*), shiny-leaf ceanothus (*Ceanothus velutinus*), and oceanspray (*Holodiscus discolor*). Impact on range here could be adverse, because none of these species have any forage value, and livestock will not feed on them unless they are under starvation conditions.

Steepness of the topography in parts of both units is such that much if any increase in beneficial forage would be unavailable to livestock.

Public health hazard.--Larvae of many species of Lepidoptera including the tussock moth possess urticating hairs (James and Harwood, 1969). These hairs are hollow and contain a chemical substance similar to but less toxic than that secreted by wasps or bees. This substance acts as a strong sensitizing agent causing allergic reactions on repeated contact resulting in hives in sensitive persons. The symptoms also may resemble those of hay fever and asthma (Press, 1973).

In some instances irritation appears to be largely due to a mechanical effect. Under certain conditions of heat, perspiration, and skin friction, the tiny hairs act as irritants in persons without allergic propensities (Press, 1973).

When larvae molt, numerous tiny barbed hairs are shed with the skin. Cocoons of pupated larvae as well as adult moths possess these hairs. Hairs are dispersed by the wind, and when in contact with skin of the neck, face, hands, and other exposed body parts, produce severe dermatitis. Ingestion or inhalation of hairs may cause internal disturbances (James and Harwood, 1969).

Eggs of the tussock moth are also covered by these hairs. When trees are felled during salvage operations in infested timber, hairs become airborne and get on the skin and into the eyes and nose of the faller.

Entomological Evaluation Summary

(Abstract of Tunnock and Livingston, 1974. See Appendix C.)

An epidemic of Douglas-fir tussock moth was detected in northern Idaho in 1972. In 1973, aerial surveys showed that nearly 100,000 acres contained various degrees of visible defoliation. An egg mass survey of five reporting units made in the fall of 1973 determined potential for damage in 1974. Based on new egg mass densities and new to old egg mass ratios, damage is predicted to be sufficiently high to warrant control on 34,138 acres in the Coeur d'Alene unit, 64,779 acres in the St. Joe unit, 4,433 acres in the Clearwater unit, 4,762 acres in the Craigmont unit, and 33,501 acres in the Nezperce unit. Total acres qualifying for treatment are 141,613. In addition, some damage might occur on 81,554 acres within the five units. These areas will be further evaluated to determine if they qualify for treatment.

II. ENVIRONMENTAL IMPACTS

The two materials being applied in this pilot control project are naturally occurring organisms. They are almost ubiquitous and probably already occur at low levels in the proposed treatment areas. The virus, almost predictable, will eventually (in 2 to 5 years) build up naturally to much higher levels per acre in the infested areas than is to be applied. This has happened during each previously documented outbreak. No environmental effects, other than to the tussock moth, have been observed as a result of high incidences of this particular virus in an area.

Safety testing of both materials have shown them to be remarkably specific. Dipel's activity is restricted to larvae of the Order Lepidoptera (butterflies and moths) whereas the virus affects only three known species of tussock moths.

Dipel has been found effective and environmentally safe for controlling many forest pests. It is currently registered for use against spring and fall cankerworm, Great Basin tent caterpillar, fall webworm, California oak moth, red-humped caterpillar, gypsy moth, and elm spanworm. In addition it is also registered for use against many agricultural pests. It is considered safe enough by the Food and Drug Administration to be exempt from all tolerances; i.e., food crops treated with Dipel can be eaten the same day they were treated regardless of residue levels.

The virus has not been registered for insect control because it is still in the experimental category.

No degradation of air, soil, or water quality is expected as a result of the proposed project. No phytotoxicity has occurred with the proposed applications.

Both materials have been submitted to stringent safety evaluation in relation to other life forms. Results of these tests follow:

Dipel.--(This section taken directly from Background Document on Bt. Abbott Laboratories Report, Appendix D.)

1. Acute Mammalian Studies

   a. Thuricide was administered to rats in a 33 percent suspension in water with carboxymethyl cellulose as a thickener. The dose was placed in the stomach by means of a ball-tipped hypodermic syringe. Doses up to 24 grams of Thuricide ($2x10^{12}$ spores)/kilogram were administered to groups of 10 rats. The animals were observed for 1 week. No fatalities occurred. No outward symptoms of toxicity. Gross and histological examination of tissues showed no differences from tissues of control animals.

Eighteen humans ingested 1 gram of Thuricide daily for 5 days. Complete physical and laboratory examinations were given prior to the experiment, at the end of the 5-day ingestion period, and 4 to 5 weeks later. Physical examinations included detailed history and records of height, weight, temperature, blood pressure, respiratory rate, pulse rate immediately after exercise and 30 and 60 seconds thereafter. Evaluations were made of genitourinary, gastrointestinal, cardiorespiratory, and nervous systems. Laboratory tests included routine urinalysis with qualitative and quantitative urobilinogen determinations (when indicated), complete blood count, sedimentation rate, blood urea N, glucose, bilirubin, and thymol turbidity tests. All subjects remained well during the course of the experiment. All laboratory findings were negative.

Dipel was fed to groups of 10 mice (16 - 25 grams) at the rate of 10 grams/kilogram (1.5×10^7 I.U.). No mortality occurred. LD_{50} was beyond 10 grams/Kg.

Dipel was fed to three female mongrel dogs. Dosage was 400 mg/kg (6×10^6 I.U./Kg). The animals were symptom free during the 48-hour observation period.

b. Dermal

Dermal effects of Bt were tested by application to shaved flanks and bellies of albino rabbits. Dosages used ranged from 20 percent suspensions to 50 mg/animal. After application half of the treated skin was abraded while the other half was left intact. Readings were made at 24, 48, and 72 hours in one test and up to 3 weeks in another. Other than local, mild erythema, no ill effects were noted in any test animal.

In another study dermal application to albino rabbits was made to test allergenicity response. Ten sensitizing doses were applied every other day for 3 weeks. Readings were made 24 hours after each application of Bt. Two weeks after the 10th application a challenge dose was applied. Only slight erythema and edema were noted. No allergenic response was elicited.

Allergenicity was also tested with guinea pigs following the procedure of Draize. No allergenic response was noted.

c. Inhalation

Bt inhalation studies were conducted on mice, rats, guinea pigs, and human volunteers.

In one test with mice, the animals were exposed to 10 grams of Bt powder for 15 minutes. Dosages were applied four times over a period of 6 days. No ill effects were noted and gross pathology was negative.

In other tests with rats and guinea pigs, exposure to a 10 percent Bt preparation for 10 minutes, no fatalities were recorded for the 1-week observation time. Dyspnea (discomfort) was noted but recovery was rapid. The animals showed normal weight gain.

Five human volunteers inhaled 100 mg. Bt powder daily for 5 days. Complete physical examinations prior to the test, immediately after the test, and 4 to 5 weeks later showed no abnormal conditions in the test subjects.

d. Eye irritation

Ocular irritation with Bt was tested in albino rabbits. 0.1 cc of a 20 percent suspension was instilled in each eye. One eye was immediately rinsed with isotonic saline. Six animals were tested. The eyes were examined immediately, after 3 hours, and 24 hours, and every 24 hours until they appeared normal. Slight redness of the eyelids was noted at 3 hours and 24 hours. Eye irritation disappeared in 48 hours.

2. Subacute and chronic studies

Bt daily administered as the spore-crystal complex to rats for 3 months at per os rates of 25, 100, and 400 mg/kg produced no main function disorders or organ damage. Similar results were obtained in dogs fed 6, 25, and 100 mg/kg for 3 months.

3. Other studies

a. Birds

Avian and fish toxicity tests have been conducted with several Bt preparations.

One long-term study with six New Hampshire Red laying hens was conducted over a 23-month period. The hens received a daily dose of Bt ranging from 0.5 to 10 grams

per bird. Results showed no allergic response, other illnesses, or variations in the expected egg production of the hens on test diet. There was no significant differences between the test birds and the birds used as controls.

In a 9-week oral toxicity test administrerd to 24 groups of 10 chicks each, no significant differences of any kind were noted between the test and control groups of chicks.

Seventeen pheasants and two partridges, all about 6 weeks old, were divided into two groups. One group was fed 1.0 gram of Bt/bird/day in two gelatin capsules. The control groups were fed two empty gelatin capsules daily.

No deaths or symptoms of respiratory, alimentary, or other disturbances were noted in the Bt fed group. Two pheasants in the control group died of trauma (due to handling). Birds in both groups exhibited feather color and pattern, bearing, and weight gain that is expected in similar groups of birds in nature.

It was concluded that no difference in behavior or development of test birds resulted from the ingestion of 1.0 gram of Bt spore-crystal complex per bird per day when compared to the control group.

In an oral acute toxicity study conducted with Bt in young adult bobwhite quail showed the acute oral medial lethal dose to be in excess of 10 gm/kg body weight. Five male and five female quail were fed 10 gm/kg by gavage. A similar group was fed distilled water as control.

The test period was 21 days. At the end of the test period all animals were sacrificed and subjected to a gross pathological examination. No pathology attributable to the test material was found. Growth rate was similar in the test and control groups.

b. Fish

Several acute fish toxicity tests were conducted with Bt preparations.

A 4-day fish toxicity study was conducted with Bt employing rainbow trout and bluegills as test animals. Two groups of 10 fish each were placed in water containing Bt at concentrations of 560 and 1,000 p.p.m. As a check each lot of experimental fish was challenged with a reference pesticide, pp^1-DDT. None of the trout or bluegills

exposed 96 hours to Bt at concentrations of 560 and 1,000 p.p.m. died. The 96 median tolerance limits (TL_{50}) of pp[1]-DDT for the same lots of fish were calculated to be 0.024 p.p.m. for trout and 0.032 p.p.m. for bluegills.

In another test with rainbow trout, results showed no acute effects on 2.5-inch trout exposed to 1 to 10 mg Bt/Kg water.

Larger rainbow trout--4 inches in length--were exposed to Bt at concentrations of 100 to 1,000 p.p.m. for 14 days. No deaths or symptoms of alimentary or behavioral disturbances were evident.

In a test with juvenile Coho salmon (1.6 inches in length), Bt was about 1/30 as toxic as DDT. The tests ran for 168 hours with concentrations of 8 to 406 mg Bt/1 water.

The 48-hour median tolerance limit of the Bt was about 50 mg/1, whereas that for DDT was 0.08 mg/1 water.

c. Effects on beneficial insects

(1) Bees

Numerous tests with several serotypes of Bt showed no deleterious effects where practical dosages were used. Heat stable exotoxin is poisonous to bees as is high concentrations of spores.

(2) Silkworm

This domesticated insect is highly susceptible to Bt particularly var. *thuringiensis*. Because of this, it is illegal to disseminate foreign preparations of any pathogen in Japan.

Virus.--(This section was taken directly from "Production, Safety Evaluation, and Infecticity Titer assay of a Nucleopolyhedrosis Virus Preparation for Field Application." Pacific Northwest Forest and Range Expt. Station Progress Report. Appendix E.)

Safety Evaluation

Toxicity-pathogenicity study

In planning for virus mass production and field application, and with the cooperation of the Agricultural Research Service, the Fish and Wildlife Service, and Hazleton Laboratories, we completed extensive

toxicity-pathogenicity tests with the active viral ingredients. The methods used for our tests were standard procedures as recommended for testing water, milk, foods, drugs, and pesticides.

All of the following tests gave negative results:

(1) Acute oral toxicity tests

(a) Mallards
(b) Pheasants
(c) House sparrows
(d) Rats
(e) Albino rabbits
(f) Mule deer

(2) Acute eye application

(a) Albino rabbits (Draize test)

(3) Acute dermal application

(a) Albino rabbits

(4) Acute intramuscular injection

(a) Albino rabbits

(5) Acute intradermal injection

(a) Guinea pigs

(6) Primary skin irritation

(a) Albino rabbits

(7) Skin sensitization study

(a) Guinea pigs (Landsteiner technique)

(8) Chronic feeding test

(a) Honey bees (see Knox, 1970)

(9) Tissue culture studies

(a) Bluegill (BF-2)
(b) Bullhead (BB)
(c) Minnow (FHM)
(d) Bullfrog (FT)
(e) Rainbow trout, embryonic (RTF-1)
(f) Rainbow trout, subadult (RTG-2)

The tissue culture study (in cooperation with Dr. Kenneth E. Wolf, of the Eastern Fish Disease Laboratory) proved that six eurythermic cell lines are totally refractory to nucleopolyhedrosis virus. Not only did we find that the virus does not replicate nor produce cytopathic effects in the cultured cells (even after forced adsorption by the method of Weiss and Dressler), but we also proved that it is incapable of interference, when the cells were subsequently challenged with infectious pancreatic necrosis virus, following exposure to nucleopolyhedrosis virus.

The results of our toxicity-pathogenicity testing program indicate that there is no reason to suspect that our preparation is unsafe for use as a pesticide in the forest environment. A summary of our tests was submitted to the virologists of the California Department of Public Health. The virologists reviewed our material and concurred that there should be no risk to human health from the use of the viral preparation as a microbial insecticide against the Douglas-fir tussock moth.

III. FAVORABLE ENVIRONMENTAL EFFECTS

Pilot testing of the two microbial agents, *B. thuringiensis* and the polyhedrosis virus, if successful, may lead to their registration for tussock moth control. Registration and use of microbial agents for control would avoid introduction of chemicals into the environment and preclude any possible adverse pesticide effects.

IV. ADVERSE ENVIRONMENTAL EFFECTS

Subsequent tree killing by bark beetles (primarily *Scolytus* and *Dendroctonus* spp.) is likely to occur. Large numbers of Douglas-fir and true fir will be left in a weakened condition; this provides an ideal habitat for bark beetle population buildup. Potential losses cannot be predicted precisely, but they could be substantial.

Some of the affected area may require artificial reforestation. Suitable tree seed and planting stock are in short supply; there may be a considerable time lag in putting some of the areas back into tree production.

Fire hazard will temporarily increase in many areas with the potential for serious environmental effects.

V. ALTERNATIVES TO THE PROPOSED ACTION

The following alternatives to the proposed action have been carefully evaluated -- no action, accelerated salvage, and direct control using chemicals. None of these appear reasonable or viable for the reasons given.

No action (normal timber sale-salvage activity)

This course of action would result in the following:

1. A loss of the majority of insect-killed timber not already committed for harvesting during the next 2 years.

2. A long-term reduction in allowable cut on National Forest lands to compensate for mortality and growth loss caused by the tussock moth.

3. A serious disruption of the future flow of timber products from the private lands involved.

4. A reduction in Federal in-lieu-of-tax payments to counties resulting from reduced timber values now and reduced future harvesting.

5. Additional expenditures for fire protection because of increased hazard created by fuel type changes.

6. A long-term impairment of recreation and esthetic values.

7. An increase in water yield for on-site and downstream use by stockmen and farmers.

8. A long-term gain in forage, increasing the carrying capacity of the range lands. This would result in additional in-lieu-of-tax payments to counties which would help offset the decrease in revenue caused by reduced timber harvesting.

The adverse results outweigh the few possible benefits. The consequences of doing nothing in an area where managed forests provide an orderly flow of timber to industry-dependent communities are highly undesirable and cannot reasonably be accepted.

Accelerated salvage

This course of action would result in the following:

1. A short-term increase in the amount of timber harvested. In the attempt to salvage as much dead material as possible, there would probably be an increase in wood-processing activity in the local area for 2 to 3 years as compared to normal activity.

2. A reduction in Federal in-lieu-of-tax payments to county governments caused by reduced stumpage prices. The value of dead timber salvaged within 2 years is about $33 per thousand board feet less than green timber.

3. The complete loss of approximately one-half the total volume of tussock moth-killed timber. This would be primarily commerical timber volumes that could not be economically harvested or that are now located within the boundaries of roadless areas. Lack of sufficient mill capacity within reasonable distances is also a factor contributing to inability to use all of the anticipated tree mortality.

4. A long-term reduction in the allowable cut on National Forest lands to compensate for the mortality and growth loss caused by the tussock moth.

5. A long-term reduction in Federal in-lieu-of-tax payments to affected county governments resulting from reduced Federal harvesting.

6. A disruption of the future flow of timber products from the private land involved.

7. An increase in total water yield, especially during periods of high runoff. Much of this would be available for downstream use by stockmen and farmers.

8. A gain in forage and browse plants which would be available to both domestic and big game animals. The amount of revenue received from grazing would be increased.

9. Additional long-term expenditures by Federal, State, and private agencies for fire protection to compensate for the increased hazard created by fuel type changes.

10. Expenditures for the rehabilitation of fish and wildlife habitat that would be impaired by the accelerated salvage logging activity. Some long-term habitat losses are also likely to occur.

Accelerated use of temporary roads would increase dust, traffic safety, air and water pollution, and maintenance problems. Soil disturbance would occur due to road building, road maintenance, and the accelerated logging operations.

Maximum utilization of available resources would be needed to carry out the proposed action. Much needed timber cultural work would have to be postponed because of manpower and time constraints.

Location and size of the cutting units dictated by the tussock moth would result in an adverse impact to big game due to the loss of suitable cover type over areas too large in size for the animals.

Direct control using chemicals

Zectran.--Zectran (carbamate) is presently registered for spruce budworm (eastern and western) and jack pine budworm. Based upon laboratory tests, Zectran is about three times more toxic to western budworm than to the tussock moth. Zectran is about one-half as toxic as DDT to the tussock moth. Zectran has a short residual life, and shows little residual insecticide activity after 48 hours. The chemical is not likely to be toxic to tussock moth larvae which hatch 2 or more days after spray application, thus two sprays would be needed.

Under forest conditions with presently available spray equipment, a spray deposition average of about 20 percent can be expected. For tussock moth outbreaks, a population reduction of at least 90 percent must be achieved to prevent unacceptable damage to host trees. Although exceptional spray deposition was obtained when Zectran was applied at the 0.15 pound/gallon/acre rate, the chemical did not kill enough larvae to prevent unacceptable damage to trees in the test plots. Doubling the dosage to 0.3 pound/gallon/acre in a single application against larger and older larvae did not satisfactorily improve the results.

Because of the variability in results and unsolved application problems, further testing of Zectran is necessary before recommendations can be made to proceed with a large-scale operational control project.

Stabilized pyrethrins.--Pyrethrins (botanical insecticides) are registered for use on many household, public health, and agricultural insect pests. They have not yet been registered for aerial application against forest insects. Small-scale field experiments have been conducted against several forest insects including the tussock moth.

In a field test against the tussock moth by the USDA Forest Service Insecticide Evaluation Project (IEP), Berkeley, California, stabilized pyrethins at 0.1 pound/gallon/acre caused 76 percent larval mortality. Spray deposition averaged only 14 percent. This level of population reduction is marginal for tussock moth suppression. Acceptable mortality might have been achieved if spray deposition had been good. Further field tests and pilot tests are necessary before pyrethrins can be recommended for operational use.

Carbaryl (Sevin).--Carbaryl (carbamate) is registered for a number of forest insects, but it is not registered for tussock moth. It is registered against the related gypsy moth, and is well known to be effective in the field on this insect at one-half to one pound/acre. Data from laboratory tests performed by USDA Animal Plant Health Inspection Service and by IEP indicate that carbaryl is over 16 times more toxic to gypsy moth than to tussock moth.

Carbaryl is known to have several days of residual insecticidal activity and appreciable stomach activity.

The Idaho Department of Public Lands (IDPL) field tested carbaryl in 1973. These tests were aimed at low population levels (about 60 larvae per 1,000 square inches of foliage) before noticeable defoliation occurred. In spray blocks, 86 percent of the trees suffered less than 10 percent defoliation as opposed to only 38 percent of the trees in the check blocks suffering less than 10 percent defoliation. Under these conditions, carbaryl shows enough foliage protection to warrant further testing.

A great deal is known about the environmental impact of carbaryl. Its use in forested areas has not been shown to cause unreasonable adverse effects.

Trichlorfon (Dylox).--Trichlorfon (organic phosphorous insecticide) is registered for use on a wide variety of crops, ornamental trees, and flowers. It is registered for aerial application in a ULV formulation against the gypsy moth.

Trichlorfon was field tested against the tussock moth as an aerial spray in 1973 by IEP. Results were promising, but not conclusive. It is about one-third as toxic as Zectran by topical application. Indications are that trichlorfon has an appreciable residual insecticidal activity since it remains toxic to gypsy moth for several days.

Trichlorfon is effective against the gypsy moth in the field at 1 pound/acre. Data from topical application to gypsy moth and tussock moth indicate that trichlorfon is about twice as toxic to the tussock moth.

IEP tests indicate that other than a temporary increase in drift of aquatic insects at 1 pound/acre, trichlorfon has no observable adverse effects on nontarget organisms. An aerial test on tussock moth appears warranted.

DDT (dichlorodiphenyltrichloroethane).--DDT is a chlorinated hydrocarbon insecticide that has a history of broad spectrum insect control. It is the only insecticide proven effective against the tussock moth in past outbreaks. No adverse environmental effects were reported from the

treated areas, but it is possible that such effects were overlooked. The USDA Forest Service recognizes the possible hazards associated with applying DDT in the environment and believes that alternative control methods should be developed.

A comprehensive analysis of the effects of DDT on the environment is included in an Environmental Impact Statement filed with the Council on Environmental Quality in March 1974 and will not be covered in this assessment.

VI. RELATIONSHIP BETWEEN LOCAL SHORT-TERM USES OF MAN'S ENVIRONMENT AND THE MAINTENANCE AND ENHANCEMENT OF LONG-TERM PRODUCTIVITY

The policy of the USDA Forest Service is to use only environmentally acceptable pest management practices. There is currently no registered or proven environmentally acceptable insecticide available for the control of the tussock moth.

Most timber stands in the infested area are managed on a sustained yield basis. As the goal of forest managers is the perpetual, even flow of timber to timber-using industries, the local short-term uses and the maintenance and enhancement of long-term productivity are much the same. Catastrophic losses of timber to the tussock moth disrupts the sustained yield of timber and seriously reduces the productivity of the timber stands for many years.

Many other forest resources are abundantly produced and utilized on a sustained-yield basis in the infested areas. Annually thousands of people use the forest for a variety of recreational experiences. The impact of the tussock moth is probably detrimental to most of these forest resources.

The Forest Service recognizes the possible hazards associated with applying DDT in the environment and believes that environmentally acceptable control procedures are needed to manage the tussock moth in such a way that both short-term and long-term production of forest resources will be optimized. In the future, these procedures could be used before outbreaks exceed economic thresholds. The prospects of developing acceptable suppression tools for use against the tussock moth are good. The proposed pilot testing of the two microbial agents will contribute to this development. Reliable outbreak prediction methods are being developed so that control can be recommended in the early stages before significant damage occurs.

VII. IRREVERSIBLE OR IRRETRIEVABLE COMMITMENT OF RESOURCES

There will be no irreversible or irretrievable commitments as a result of the proposed action.

VIII. SUPPORTING RESEARCH AND DEVELOPMENT PROGRAMS

The problem of tussock moth outbreaks can eventually be solved by an integrated control strategy designed to contain populations below the level where tree damage occurs. Strategies might include a combination of methods involving forest management practices, continuous population surveillance for prediction and the timely amendment of biological agents or chemicals for population suppression where needed. The development of an effective integrated control strategy obviously requires considerable knowledge of insect population behavior, tree damage and the regulatory factors commonly operating in natural tussock moth systems.

Before 1960, the nature of tussock moth research was largely empirical and descriptive. Studies were usually fragmentary and somewhat periodical, often coinciding with the high interest generated during times of outbreaks. As a rule, when outbreaks subsided research interests also waned. However, since the early 1960's, because of the emphasis placed on developing alternative methods to chemicals for insect control, tussock moth research has been conducted on a more continuous basis.

There has been a renewed interest in tussock moth recently due to the current rash of outbreaks in the Northwest. This has been followed by a strengthening of ongoing research and the initiation of a number of new investigations.

Until recently, most of the significant tussock moth research in the United States has been conducted by entomologists at the University of California and by the U.S. Forest Service at its laboratories at Corvallis, Oregon, and Berkeley, California. Investigators at the University of California have been concerned largely with developing egg sampling methods and studying natural enemies of the tussock moth. Forest Service research at the Pacific Northwest Forest and Range Experiment Station consists of a comprehensive program involving tussock moth population dynamics, forest impact, and biological control. Main objectives of the program have been to develop an integrated pest management system for tussock moth which will ultimately assist in decisionmaking and produce alternative strategies for control. Through this effort much headway has been made toward the development of a population-impact model which will enable tree and stand damage to be predicted from sampling information.

New research projects begun in the last year due to the current frequency of outbreaks have the potential for adding new breadth to our knowledge of tussock moth. Most significant of these is the cooperative effort of the Pacific Northwest Forest and Range Experiment Station and the Oregon Graduate Center to chemically identify the Douglas-fir tussock moth pheromone. Related pheromone studies are also being conducted by Boyce Thompson Institute, Grass Valley, California, and by the Canadian Forestry Service, Sault Ste. Marie, Ontario. The availability of

a synthetic pheromone would improve methods of early detection of outbreaks and possibly offer alternative control opportunities. Graduate students at the University of Washington, Washington State University, and Oregon State University have recently undertaken a variety of thesis projects involving the effects of site and weather on outbreaks, infestation of defoliated trees by secondary bark beetles and the detection of outbreaks by high elevation and satellite imagery. All of these new studies give needed support to ongoing research programs.

Development of Microbial Insecticides

Formulations of two microbial agents, one a nuclear polyhedrosis virus and the other the bacterium, *Bacillus thuringiensis*, were tested for effectiveness against the Douglas-fir tussock moth in 1973 by researchers with the Pacific Northwest Forest and Range Experiment Station, Corvallis, Oregon. Various treatments were applied by helicopter to small, 20-acre field plots on the Wallowa-Whitman National Forest about 24 miles northeast of Enterprise, Oregon.

Preliminary results showed virus applications were highly effective in reducing tussock moth larval numbers. This in turn resulted in significant foliage protection with foliage loss being mainly confined to the new flush of needles. The bacterium provided comparable reductions in the number of larvae and in foliage protection when applied in an experimental molasses formulation, but was considerably less effective when applied in a Biofilm formulation. Tussock moth larval numbers remained at a high level on the untreated plots until they completely defoliated a high proportion of the trees.

Final evaluation of the 1973 field tests is not complete, but the results to date indicate that both *Bacillus thuringiensis* and the virus hold very good potential for control of the Douglas-fir tussock moth. The work completed to date, however, also indicates that both materials are still too early in their development and contain too many unsolved problems relating to their use and application to be considered for operational use by 1974.

At present, the two materials lack experimental confirmation, and have only been shown effective when applied to 20-acre plots. To progress from these limited plots to an operational basis will require considerable additional field testing. Similarly, problems remain to be solved in the development of mixing and handling techniques and in the development of a carrier formulation.

The only logical recommendation that can be made at this time is that an expanded research program be undertaken with the goal of having both materials fully tested and registered by 1975.

Development of Chemical Insecticides

Scientists with the Insecticide Evaluation Project, Pacific Southwest Forest and Range Experiment Station, Berkeley, California, have been screening and testing numerous insecticides to replace using DDT against many forest insect pests including the Douglas-fir tussock moth.

Formulations of four chemical insecticides, Zectran, Dylox, Sevin 4-Oil, and Bioethanomethrin were tested for effectiveness against the Douglas-fir tussock moth in 1973. None of these four chemicals was effective enough under the conditions of these tests to advance to the pilot testing stage.

Additional experimental field testing will be needed for all four candidates under different spray strategies before their potential can be adequately evaluated.

Preliminary laboratory studies with a new experimental compound that blocks the formation of chitin has considerable promise against the Douglas-fir tussock moth.

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X. APPENDIX

A. Work plan for pilot tests

B. History of past epidemics and suppression activities

C. Entomological evaluation

D. Background data on *Bacillus thuringiensis*

E. Background data on the nuclear polyhedrosis virus

WORK PLAN

DOUGLAS-FIR TUSSOCK MOTH PILOT PROJECT USING MICROBIAL INSECTICIDES

MAY 1974

STUDY PLAN

A PILOT CONTROL PROJECT AERIALLY APPLYING A NUCLEOPOLYHEDROSIS VIRUS AND *BACILLUS THURINGIENSIS* TO DOUGLAS-FIR TUSSOCK MOTH POPULATIONS IN IDAHO 1974

Prepared by Harold E. Dawson — March 13, 1974
Chairman, Interagency Douglas-fir Tussock Moth Pilot Project Task Force — Date

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for Director, Pacific Northwest Forest & Range Experiment Station — Date

Russell K. Smith — 5/3/74
Director, Forest Pest Control Washington Office — Date

U.S. DEPARTMENT OF AGRICULTURE - FOREST SERVICE
Division of State and Private Forestry
Northern Region Missoula, Montana

TABLE OF CONTENTS

	Page
INTRODUCTION	1
OBJECTIVES	2
METHODS	2
Treatments	2
Criteria for Test Plot Selection	5
Natural Virus Level Determination	5
Sampling Design	6
Description of Sample Trees	9
Timing of Application and Development Sampling	10
Population Sampling	10
Laboratory Activities	11
Mixing	11
Spray Application (Equipment and Timing)	13
Collection and Assay of Field Samples	13
Spray Deposit Sampling	13
Environmental Monitoring	14
Assessing Foliage Retention	14
Data Analyses	16
INFORM AND INVOLVE	17
COOPERATION	17
ORGANIZATION	19
FINANCIAL PLAN	21
REFERENCES CITED	23
APPENDIX	24

INTRODUCTION

The Douglas-fir tussock moth, *Orgyia pseudotsugata* McD., periodically reaches epidemic levels in the Northwest. Detailed histories of outbreaks in the Northern Region, Oregon, and California are discussed by Wickman et al. (1973) and Tunnock (1973). Past outbreaks have usually persisted for 2 to 4 years at which time they die out due primarily to epizootics of a nucleopolyhedrosis virus.

With the advent of DDT in the mid-1940's came the first promising insecticidal treatment for broad-scale Douglas-fir tussock moth control. In 1947 over 400,000 acres of tussock moth infested forests in Idaho were aerially sprayed with 1 pound of DDT in 1 gallon of fuel oil per acre. Another 120,000 acres of tussock moth infested forests were sprayed with DDT in Idaho in 1965 (Tunnock 1973).

As the undesirable properties of large-scale DDT spraying became known, the Forest Service discontinued spraying with this insecticide. DDT spraying was terminated in Region 1 in 1965. Since then work has been conducted to develop environmentally acceptable materials for Douglas-fir tussock moth control. At least 12 chemical insecticides and two microbial agents have been either laboratory or laboratory and field tested against the tussock moth (Anon. 1973). Two microbials, a nucleopolyhedrosis virus and the bacterium *Bacillus thuringiensis* Berliner, have shown the most promise of the materials field tested to date.

The most recent field tests of these materials against the Douglas-fir tussock moth were conducted in eastern Oregon in 1973. Various treatments were applied by helicopter to 20-acre field plots. Preliminary results showed that larval populations were reduced from 74.5 to 99.9 percent depending on the formulation (Thompson and Markin 1973). As a result of these successful field tests the next step in the developmental process of these materials is a pilot control project.

The current tussock moth outbreak in northern Idaho is believed to provide the elements needed for a definitive pilot project with virus and bacteria. The elements as described by Stelzer and Neisses (1973) are:

1. Relatively high tussock moth larval densities.

2. A population in the release phase of the outbreak cycle (as defined by Wickman et al. 1973).

3. A population with a very low level of natural virus associated with the overwintering eggs.

OBJECTIVES

The objective of this project is to separately evaluate the effectiveness of an aerial application of Dipel (*Bacillus thuringiensis* or *B.t.*) and a nucleopolyhedrosis virus in reducing Douglas-fir tussock moth larval populations when applied under normal operational conditions.

Secondary objectives include:

1. Determine the extent of foliage protection achieved by the treatments.

2. Investigate the correlation of spray deposit with larval mortalities.

3. Determine the incidence of parasitism of the Douglas-fir tussock moth under the limitations of the sampling schedule.

METHODS

Treatments

The pilot control project will be conducted at two locations, Coeur d' Alene Mountain south of Coeur d'Alene, Idaho, on the Idaho Panhandle National Forests (fig. 1) and Lookout Butte, south of Lowell, Idaho, on the Nezperce National Forest (fig. 2). Both materials will be applied at each location.

An aerial application of virus and bacteria, formulated as shown in Table 1, will be applied to both areas at the rate of 2 gallons per acre.

Table 1.--Composition of proposed spray formulations to be applied per acre

Active ingredients	Adjuvants (gallon)	NaOH (grams)	B.S.F.[1/] (grams)	Sun screen (pound)	Water (gallon)
Douglas-fir tussock moth virus (100 billion polyhedra)	C.I.B.[2/] (.5)	-- 26.4	-- 7.6	IMC 90-001 (1.0)	-- 1.5
Bacillus thuringiensis (1 pound)	C.I.B. (.5)	-- --	7.6 --	-- --	1.5 --

1/ Brilliant sulfur yellow dye.

2/ Cargills insecticide base (molasses).

Fig. 1
Coeur d'Alene Mountain infestation area,
site of proposed spray plots.

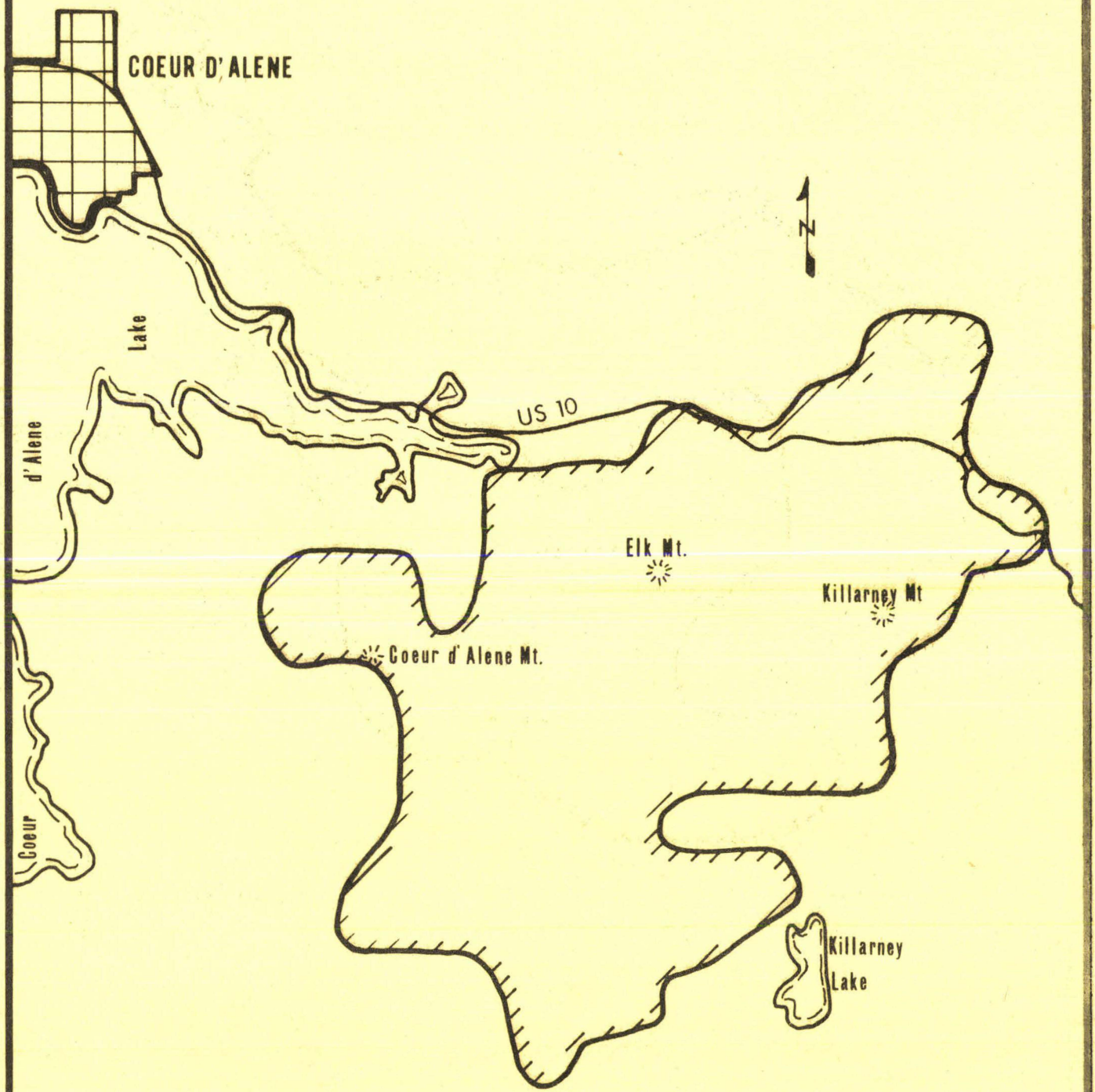
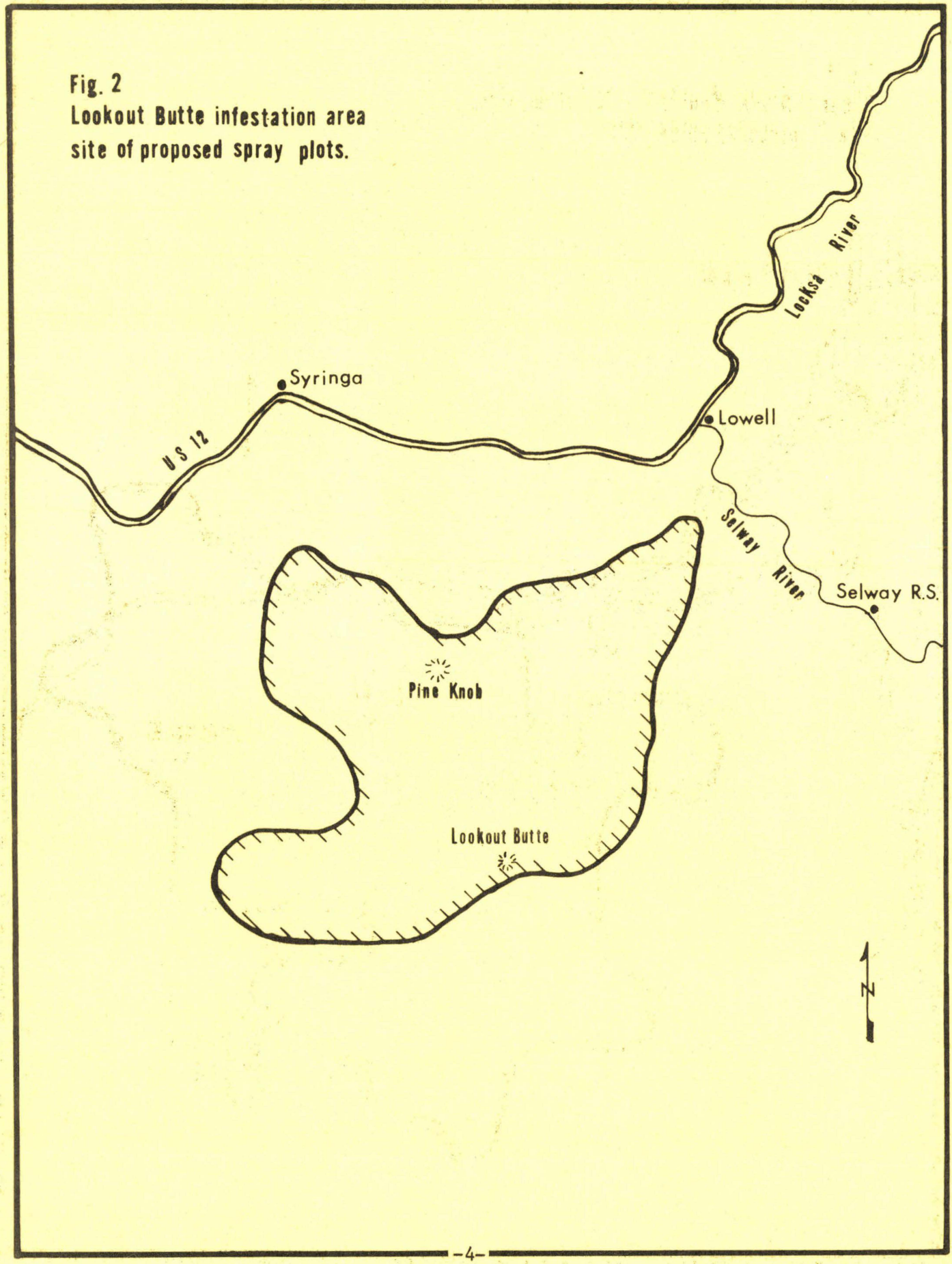


Fig. 2
Lookout Butte infestation area
site of proposed spray plots.



A portable pH meter will be used to monitor the pH of the spray suspension during the mixing operation. The pH should remain near 6.6 ± 0.3. Since the pH of the molasses solution may vary from batch to batch, additional amounts of NaOH pellets may be required to stabilize the pH at the desired level.

The two areas will each be divided into nine plots (three virus, three bacteria, and three controls). Plots will not necessarily be the same size or shape. Natural boundaries, i.e., creeks, ridges, type changes, burns, clearcuts, etc., will dictate their size. A minimum of near 1,000 acres and a maximum of about 5,000 acres per plot have been set as guidelines.

Within each plot an 800-acre area will be identified as a sampling zone. All sample trees for measuring tussock moth population levels will be located within this area.

Enough virus is available to treat only 6,500 to 7,500 acres. Hence, the six virus plots will be about 1,000 acres in size.

Criteria for Test Plot Selection

Plots must have good accessibility and somewhat natural boundaries. Tussock moth population levels and current defoliation should be more or less uniform among the plots. The sampling portion of the plots will be located a sufficient distance from the next plot to minimize contamination by spray drift. Distances will vary with the terrain and wind patterns.

Natural Virus Level Determination

Egg masses were collected from 26 sections of land within the proposed Coeur d'Alene Mountain treatment area and from 22 sections within the Lookout Butte area as a part of the 1973 egg mass survey. These were used for the virus level determination. Five egg masses per section, if that many were available, were used. Egg masses were labeled as to collection site and placed in refrigeration at approximately 35° F.

About February 1, these masses were bisected and one-half of each mass sent to the Insect Disease Project (PNW-2203) and the other half kept in Missoula. Eggs are now being hatched and 20 to 30 larvae per egg mass are being reared on artificial diet in a single Petri dish. Fourteen days after hatching, survival of larvae from each egg mass will be determined. Dead larvae will be diagnosed for cause of death. The virus level in the overwintering population will thus be determined by early March. Egg parasitism and percent egg hatch will also be determined at this time.

If an average of 5 percent or more of the larvae from an area are infected with the naturally occurring virus, that area will be deleted as a test site. If the virus level is between 1 and 5 percent, other

factors such as tussock moth population density will have to be evaluated before the area can be accepted or rejected as a test location. If the natural virus level is less than 1 percent, the area can be considered usable in this regard.

If virus levels are too high in the proposed areas, alternate test sites will be searched for in Idaho, Washington, or Oregon. Possible alternative test sites will be identified by mid-March following the laboratory virus level determinations.

Sampling Design[1/]

An analysis of variance was made from the prespray Douglas-fir tussock moth data from a test of Sevin-4-oil conducted by the Idaho Department of Public Lands during 1973 to determine the most desirable number of branches per tree, trees per cluster, and clusters per area to estimate a sample mean (Table 2). A nested design was chosen to measure the variability between areas, clusters, trees, and branches. The trees were to be selected in clusters of 10, although often the size of area enclosing the 10 trees was quite variable.

Table 2.--Analysis of variance for branches, trees, clusters, and areas

Source	Degree of freedom	Sum of squares	Mean squares
Total	639	2,497,470	3,908.40
Areas	3	779,202	259,734.00
Clusters	12	294,276	24,523.00
Trees	144	729,473	5,065.79
Branches	480	694,518	1,446.91

An estimate of the sample variance using different combinations of branches per tree, trees per cluster, and clusters per area can be used to determine optimum sample size for the 1974 tussock moth pilot projects.

The estimated sample variance can be calculated in the following formula (Snedecor 1956):

1/ The experimental design was chosen by consultation with Dr. Donald V. Sisson, biological statistician, Utah State University, Logan, Utah, and Dr. Michael Marsden, Northern Fire Laboratory, Missoula, Montana.

$$\sigma^2_x = \frac{\sigma^2_b}{btc} + \frac{\sigma^2_{bt}}{tc} + \frac{\sigma^2_{btc}}{c}$$

Where σ^2_x = estimated variance of branches, trees, clusters

σ^2_b = mean square value for branches

σ^2_{bt} = $\frac{\text{mean square for trees - mean square for branches}}{\text{number of branches per tree taken}}$

σ^2_{btc} = $\frac{\text{mean square for clusters - mean square for trees}}{\text{number of branches x number of trees}}$

b = number of branches per tree
t = number of trees per cluster
c = number of clusters per area

The predicted variance (σ^2_x) using different combinations of branches, trees, and clusters is shown in Table 3. The mean prespray population in the 1973 Idaho test was 64.95 larvae per 1,000 square inches of foliage. The values of σ^2_x is the estimated variance of that mean.

Table 3.--Estimated variance for various combinations of branches, trees, and clusters

4 branches, 10 trees, 4 clusters (40 trees/area)

$$\sigma^2_x = \frac{1,446.91}{4x10x4} + \frac{904.72}{10x4} + \frac{486.4}{4} = 153.26^{1/}$$

2 branches, 10 trees, 4 clusters (40 trees/area)

$$\sigma^2_x = \frac{1,446.91}{2x10x4} + \frac{904.72}{10x4} + \frac{486.4}{4} = 162.30$$

2 branches, 10 trees, 8 clusters (80 trees/area)

$$\sigma^2_x = \frac{1,446.9}{2x10x8} + \frac{904.72}{10x8} + \frac{486.4}{8} = 81.20$$

2 branches, 8 trees, 10 clusters (80 trees/area)

$$\sigma^2_x = \frac{1,446.9}{2x8x10} + \frac{904.72}{8x10} + \frac{486.4}{10} = 68.99$$

2 branches, 5 trees, 16 clusters (80 trees/area)

$$\sigma^2_x = \frac{1,446.9}{2x5x16} + \frac{904.72}{5x16} + \frac{486.4}{16} = 50.75^{2/}$$

1/ Combination of branches, trees, and clusters used in the 1973 Idaho test.

2/ Combination of branches, trees, clusters chosen for 1974 tests.

Little is gained by taking four branches per tree compared to taking two (Table 3). More trees, rather than branches, per area will reduce the variance estimates to a greater degree. There is also an advantage in taking only two branches so that subsequent postspray sampling would not deplete desirable branch samples as rapidly as four branches per postspray sample. Postspray samples may be biased if a tree does not contain an excess of good sample branches.

The analysis also points out that more clusters per area and fewer trees per cluster would decrease the variance without increasing the number of sample trees. There is, however, a point at which a cluster should contain enough trees to assure that negative mortality for the cluster would not occur. Negative mortality may become critical if regression is used to correlate deposit with mortality.

Two other important points favoring fewer trees per cluster and more clusters are:

1. It is more difficult to obtain 8 to 10 trees in a cluster than 4 to 6 trees because of the availability of suitable sample trees in the cluster area.

2. Fewer trees per cluster would have a greater chance to receive uniform deposit because of spray coverage variability. A small cluster would be more likely to be sprayed by a single swath than a large cluster covering more area.

Based on the estimated variances and what is feasible manpowerwise, the selected sample design is to cluster samples, taking two branches per tree, five trees per cluster, and 16 clusters per area for a total of 80 trees per area. Clusters will be located at least 5 chains apart and should not occupy more than 1 acre of ground. Clusters will be located throughout the spray area. If it is impossible to cluster sample because five trees cannot be found on 1 acre, or 16 clusters cannot be located in a plot, single tree samples will be used.

The two project areas (Coeur d'Alene Mountain and Lookout Butte) will be sampled identically. If the single tree method is necessary in one area, it will be used in both. The area judged most difficult to cluster sample will be laid out first to determine if the cluster method is feasible or if the sample design will be based on single trees.

Using the same data from the 1973 Idaho tests, an analysis of variance was made assuming that the trees were not clustered in order to select a suitable number of sample trees if the single tree sampling method is needed (Table 4).

Table 4.--Analysis of variance for areas, trees, and branches

Source	Degree of freedom	Summary of squares	Mean squares
Total	639	2,497,470.0	3,908.40
Areas	3	779,202.0	259,734.00
Trees	156	1,023,750.0	6,562.49
Branches	480	694,518.0	1,446.91

Following the procedure described previously, a two-way table was constructed showing various combinations of branches and trees to estimate a variance for the population mean at 64.95 larvae per 1,000 square inches of foliage (Table 5). From this a sample size of 80 trees/plot, two branches/tree was selected if the single tree method is used.

Table 5.--Estimate of variances for different combinations of branches and trees per area

No. trees	Branches per tree				
	1	2	3	4	5
30				54.680	52.270
35			50.319	46.870	44.800 1/
40		50.064	44.030	41.014 2/	39.206
45		44.496	39.138	36.458	
50	54.516	40.047	35.217	32.811	
55	49.560	36.406	32.022		
60	45.430	33.372			
65	41.935	30.805			
70	38.939	28.604			
75	36.343	26.697			
80	34.072	25.029 3/			

1/ Sample below line shows an estimated variance which would be less than the 1973 Idaho tests data.

2/ Variance for the number of branches and trees for sample data from the 1973 Idaho test.

3/ Sample size chosen for 1974 tests and estimated variance.

Description of Sample Trees

Sample trees are defined as 30 to 50 feet tall Douglas-fir or grand fir. This height is used because of equipment limitations in sampling taller trees. Sample trees should be somewhat open grown and not shielded by larger trees. They must have sufficient foliage to support high tussock moth populations.

Timing of Application and Development Sampling

Spraying will begin when 50 percent of the larvae have reached the second instar. This will probably be in late June. Timing of application will be determined by:

1. Tagging 40 egg masses per plot prior to ecosion and examining them two or three times a week for hatch. Newly hatched larvae cluster on the mass for a day or two before they disperse. After 50 percent of the egg masses have hatched, branch samples will be collected for instar determination.

2. Two midcrown branches from 20 trees per plot will be collected every other day when 50 percent of the egg masses have hatched. These will not come from population sampling trees. Development sampling will give field crews experience using pole pruners and processing samples. Samples will be taken daily from each unit when 20 percent of the larvae are second instar. Larvae will be placed in alcohol and taken to the laboratory for instar determination by head capsule measurement.

Population Sampling

Douglas-fir tussock moth larval populations will be sampled within 48 hours prior to spraying and again following spraying at 7, 21, and 35 days, and shortly before pupation. Control plots will be sampled at the same time intervals. The population sample will be taken by extending a 24-foot telescopic pole pruner with catch basket attached into the midcrown portion of the sample tree and clipping approximately the distal 18 inches of two branches from opposite sides of each tree sample in such a way that they fall into the basket. Two branches will be collected per sample tree each sampling period. Branch samples and all contents of the basket will be carefully placed on canvas tarps for larval counting and branch measurement. The following information will be recorded at each tree:

1. Date
2. Treatment area
3. Tree number
4. Tree species
5. Branch length
6. Branch width
7. Number of tussock moth larvae
8. Crew leader

The basic population measurement unit will be tussock moth larvae per 1,000 square inches of foliage.

A survey of the egg mass population within the plots will be conducted during fall 1974. This will be to determine if the treatment effects

carry over to the overwintering population; i.e., if there are proportionately fewer egg masses in treated areas compared to untreated areas.

Sampling crews will be given special instructions and training in the field before the actual sampling is started.

Laboratory Activities

A field laboratory will be operated at both project areas. The main functions of this facility are:

1. Serve as project headquarters
2. Provide suitable conditions for laboratory rearing of larvae
3. Provide a place for diagnostic work
4. Provide accommodations for preliminary data examination

If available, 10 larvae will be collected for laboratory rearing from each sample tree during each sampling period. These will be collected by the field crews and placed separately in Petri dishes containing artificial media. The main objective is to diagnose dead larvae to determine the cause of mortality (virus, bacteria, parasites, unknown) on the 14th day after collection. In addition, any measurable differences in the parasite complex present at each sampling period will be determined.

Mixing

Virus.--The available virus preparation is unsuitable for mixing and aerial application due to the presence of body parts and hairs remaining from its preparation. This "dirty" stock material must be cleaned up a day or two (no more than 4) before mixing of the final spray formulations. Cleanup consists of mixing the freeze-dried "dirty" virus in 0.02 molar solution of Trizma 7.1 buffer; then, using a small capacity mixing tank with some form of recirculation, progressively filtering the liquid through 50-, 100-, and 150-mesh screen if a Bell 205A or Bell 206 helicopter is to be used. If a Bell 47G is used, a final filtering through a 200-mesh screen should be included. More details on virus cleanup are included in Appendix I.

Mixing sequence for virus is:

1. Fill mixing tank with water and if cold, heat or allow to stand until temperature exceeds 60° F.

2. Add brilliant sulfur yellow dye (BSF), mix 5 minutes.

3. Add sunscreen, using suction type system and mix for 20 minutes.

4. Add molasses by slowly pouring into mixture. Molasses temperature should be 70° to 80° F. for good flow ability.

5. Check pH and adjust by adding NaOH to a pH of 6.6 to 7.2 and mix thoroughly.

6. If the carrier formulation is mixed the day before use, it can be stored overnight and the virus added the next morning. Before adding the virus, the pH should again be checked and the formulation mixed again for 10 minutes.

7. The virus solution should be directly added to the carrier solution and mixed for 10 minutes before loading.

8. In case of an emergency, unused finished virus spray can be kept 24 hours if constant check is made of its pH to see that it does not exceed 7.5, but whenever possible virus should be added just before loading.

Dipel.--Steps in mixing Dipel are:

1. Fill mixing tank with water and if cold, allow to stand or heat to 60° F.

2. Add dye and mix 5 minutes.

3. Add Dipel, using suction type system and mix for 20 minutes.

4. Add molasses and mix for 20 minutes. For easy pouring, molasses should be 70° to 80° F.

Filtering.--With both Dipel and virus, mixing should be done with a recirculating system (besides mechanical agitation) and both must be filtered. If a Bell 205A or Bell 206 is to apply the material, a 60-mesh screen should be used both in mixing and in the helicopter's in-line filter. No nozzle screens are needed. If a Bell 47G is used, the mixing tank filter should be 100-mesh and the helicopter's in-line screen and nozzle screen should be 50-mesh. The helicopter in-line screen should be checked and cleaned after each load, and the nozzle screen after every other load when virus is used.

Handling.--It must always be remembered that the agents being used are living organisms and not inert chemicals and are very likely to be killed by conditions that would have no effect on chemical insecticides. Microbials, therefore, need much more care in handling and preparation. The water to be used should be checked to be certain it contains no contaminants that could affect the microbials, such as chlorine found in most cities' water supplies. Before using, all mixing and spray equipment should be thoroughly cleaned and checked to be certain they contain no detergents, chemicals, or solvents that could affect the microbials. Temperature is also very critical, and Dipel and freeze-dried virus should always be shipped and stored at termperatures less than 90° F. Both materials must also be kept completely dry until mixing. A very critical watch must also be kept during mixing and

and agitation in the helicopter to see that the temperature does not rise above 100° F. for more than a few minutes.

Spray Application (Equipment and Timing)

Spray will be applied with helicopters. The spray system will have circulation or agitation in the tank and be capable of maintaining pressures of at least 50 pounds per square inch. Boom length and nozzle configuration will be such to allow a 2-gallon-per-acre treatment with a droplet size of 150 to 200 m.m.d. Swath width at tree top height will be 50 to 100 feet at a release height of 50 feet above the tree tops, depending on helicopter type.

Spraying will be done during the early morning hours when the air is cool and still. Spraying will be terminated when winds exceed 6 miles per hour at ground level in clearings or temperatures are over 65 degrees.

An attempt will be made to spray one plot (one-third of a treatment) per day per area. The sampling zone will receive treatment priority. The remainder of the plot will be treated within 48 hours of the sampling zone.

Collection and Assay of Field Samples

One 20 ml. spray sample will be collected at the spray system nozzle immediately after application of each tank load of the suspension. Containers will be supplied. Each container shall be labeled and immediately stored in an ice chest. Biological assay of the sample and polyhedron counts will be performed at the Pacific Northwest Forest and Range Experiment Station laboratory in Corvallis, Oregon, as described by Martignoni, 1970. If there is a possibility that the spray tanks might be completely emptied in flight, then one sample should be collected at the nozzles before each application to insure that at least one set of samples will be available for study. The most important samples are those collected after the application. If available, these samples will be used for bioassay, whereas the set of samples collected before that particular application will be discarded. This redundancy is indicated in order to secure at least one set of samples per application.

A predetermined number of larvae will be inoculated with a sample from each helicopter load with a micro injector. The objective of this is to compare virulence of material going into the spray tanks with that coming out. A malfunction in a recirculation system could create sufficient heat to de-activate the pathogens.

Spray Deposit Sampling

The same trees will be sampled for insect mortality and spray deposit. The basic deposit sampling unit will be four 10- to 15-inch branches

taken from the midcrown of each sample tree, two aluminum plates 6 by 6 inches, and one white Kromekote card. The plates and white card will be positioned at ground level in the nearest open area adjacent to each sample tree. The plates and cards will be placed in the field just prior to spraying. Cards and plates will be collected immediately after spray application. Aluminum plates will be placed face to face (deposit sides together) and stored in slotted boxes for transportation. The four foliage samples will be collected from each cardinal direction of sample trees and placed in brown paper bags. Tree number, sample direction (north, south, east, west) and time of collection will be recorded on each bag.

Environmental Monitoring

Both materials to be tested have been determined to be very host specific. Dipel affects a number of lepidopterous larvae, and the virus affects only a few species of tussock moth.

Dipel is considered so safe to man that it is exempt from tolerance limits. That means sprayed food plants can be eaten the same day of spraying even though Dipel residues remain. Its oral and dermal toxicity has been tested on a number of mammals including humans, rats, mice, dogs, rabbits, and guinea pigs. No deleterious effects were found. It has also been tested on chickens, pheasants, partridge, and quail, plus rainbow trout (both immatures and matures), blue gills, and Coho salmon. Test results were negative (Anon. 1974).

No phytotoxicity has been reported with Dipel. Some earlier *Bacillus thuringiensis* formulations showed some phytotoxicity; however, this was believed to have resulted from the adjuvants rather than from the active ingredient (Anon. 1974). The virus has been safety tested on selected mammals, birds, fish, and nontarget insects with negative results (Martignoni 1972).

Because it has been well documented that these materials are environmentally safe, the only environmental monitoring planned for this pilot control project is related to the effect of the treatments on other lepidopterans. Contracts are being prepared to be offered to two graduate students, through universities, to examine these effects.

It should be remembered that both of these test materials are naturally occurring and are present at varying levels in most places.

Assessing Foliage Retention

To measure the effect of spray treatments on foliage retention, each sample tree will be rated as to degree of foliage saved. This will be accomplished by visually rating each sample tree prior to tussock moth feeding (late May or early June) and after feeding has been completed in late August. The same person will rate the same plot both times. He will be thoroughly trained in rating defoliation.

A matrix system which is quite sensitive to variation of feeding patterns and proportion of foliage by crown levels will be used.

To account for the variation in crown level feeding characteristic to tussock moth, each tree will be divided into six crown levels of equal length by using a rule with six equal parts. Each crown level will be given a numerical defoliation rating based on the following table:

Table 6.--Crown level ratings

0 - Negligible.	No feeding visible.
1 - Light.	Feeding on new growth only.
2 - Moderate.	Noticeable feeding on new and old growth (less than 50 percent defoliated).
3 - Heavy.	Conspicuous feeding on new and old growth (more than 50 percent defoliated).

Crown levels will be given weights according to their intensity of tussock moth damage. Characteristically the tussock moth defoliates a tree from the top down and by the time the lower crown is defoliated, the impact on that tree is severe and carries more weight than if just the upper crown was fed on. Weights for the tree crown levels will be assigned as 1 (upper one-sixth) to 6 (lower one-sixth). The defoliation index for each tree will be determined by summing the defoliation rating on a crown-by-crown basis (Table 7).

Table 7.--Intensity of defoliation and weighting factors

Crown levels	Negligible 0	Light 1	Moderate 2	Heavy 3	Defoliation index
Upper 1	0	1	2	3	
2	0	2	4	6	
3	0	3	6	9	
Mid 4	0	4	8	12	
5	0	5	10	15	
Lower 6	0	6	12	18	_____

The numerical value for various combinations of defoliation indexes for each cluster and area can then be tested to see if significant differences occur between mean for various treatments.

Aerial photography will also be used to measure defoliation intensities within the treatment units. False color aerial photography has been shown to be a valuable tool for assessing effects of aerial sprays

applied to suppress forest defoliators by providing estimates of foliage saved in spray blocks as compared with surrounding areas which have not been treated.

Aerial photography of each block will be obtained with Kodak Ektachrome infrared aero film, type 2443, at a scale of 1:15,840 prior to defoliation to establish a base line, and again when the tussock moth larvae have completed their feeding and defoliation is most conspicuous. Aerial photos will be examined in stereo to determine area (acres) protected by the spray application within the designated spray block and adjacent stands. Maps showing areas of foliage protection, degrees of defoliation, and zones of spray drift in relation to the spray blocks will be prepared in accordance with procedures described by Ciesla, et al. (1971).

Data Analyses

Various kinds of data will be produced by the sampling efforts in this pilot test. Some of them are:

1. Estimates of prespray and postspray larval population densities for each tree and treatment block. The estimates of population density will be made from population counts expressed as number of tussock moth larvae-pupae per 1,000 square inches of foliage.

2. Estimation of mortality (population response to the treatments) by ratio estimation; e.g., the survival rate, is given by:

$$r_i = (X_{2i}/Y_{2i})/(X_{1i}/Y_{1i})$$

Where X_{1i} and X_{2i} denote prespray and postspray insect counts and where Y_{1i} and Y_{2i} denote prespray and postspray measurements of square inches of foliage for the ith tree in each treatment area. Survival ratios will be used rather than percentage reduction *per se*, so as to provide a comparison on the same basis as actual survival counts.

3. Visual estimates classifying tree defoliation caused by tussock moth feeding.--Multiple regression techniques will be used to develop formulas to predict defoliation indexes. The two independent variables judged to contribute a major portion of the variance in the regression are (a) prespray population, and (b) condition of the tree prior to feeding or prespray tree index. Multiple regression formulas will be developed from control areas where the infestation is allowed to run its natural course. One other variable which might be entered is the level of natural virus in the area. If we are able to measure this for the different areas from our virus evaluation, we will enter it as the third independent variable.

The regression formula would appear as:

Y = BO + X1 (prespray population) + X2 (prespray defoliation index)
When Y = predicted defoliation index
BO = regression intercept
X1 = regression coefficient for prespray population
X2 = regression coefficient for prespray defoliation index

A paired T test can then be used to test significant difference between the actual and predicted defoliation indexes. If the means are significant and the predicted index mean is higher than the actual, then foliage was saved by control efforts.

4. Estimates describing a range of deposited dosage for each sample tree for each microbial treatment.--The relationships between insect mortality and spray deposits will be described by regression techniques.

5. Visual estimates classifying tree defoliation caused by tussock moth feeding:

a. Aerial photographs

b. Classification by undamaged, partly damaged, and severely damaged categories.

6. Variations in parasite populations related to the different sampling periods.

INFORM AND INVOLVE

Publicity and public involvement will be coordinated by the I&I Officer.

An environmental analysis will be made to appraise the effects of the project on all segments of the environment. This report will be available for public review.

Appropriate news releases and public meetings will be held in key areas. Groups and individuals having special interest in the project will be met with to answer project related questions.

COOPERATION

This pilot control project will be a cooperative effort between the Division of State and Private Forestry, Region 1; Aerial Application Project (PNW-2208); Forest Insect Disease Project (PNW-2203); Missoula Equipment Development Center (MEDC); and Division of Timber Management, Region 6. This study plan was prepared jointly by members from each of these groups. Publication of the results of this field test will be under the joint authorship of the appropriate cooperators.

Specific areas of responsibility follow:[2/]

1. Design, planning, and review of test. Region 1, Division of State and Private Forestry; Nezperce and Idaho Panhandle National Forests; PNW-2208; PNW-2203; and Division of Timber Management, Region 6.

2. Development and conduct of inform and involve program. Nezperce and Idaho Panhandle National Forests and Division of I&E, Region 1.

3. Preparation of appropriate environmental analyses. Region 1, Division of State and Private Forestry, Nezperce and Idaho Panhandle National Forests, PNW-2208, and PNW-2203.

4. Preliminary work on mixing and application techniques. PNW-2208 and MEDC.

5. Virus contracting and materials quality control. PNW-2203; Division of State and Private Forestry, Region 1; and Division of Timber Management, Region 6.

6. Contracting of aircraft. Region 1, Air Operations and Division of State and Private Forestry.

7. Employment of temporary personnel and selection of field laboratory site. Nezperce and Idaho Panhandle National Forests.

8. Selection of spray blocks and heliport location. Region 1, Division of State and Private Forestry; PNW-2203 and 2208; MEDC; Nezperce and Idaho Panhandle National Forests.

9. Calibration, determination of spray atomization, swath width, etc. PNW-2208 and MEDC.

10. Mixing, loading, and ground handling. MEDC and PNW-2208.

11. Spray deposit assessment. PNW-2208.

12. Meteorological support. U.S. Weather Service and MEDC.

13. Laboratory operations. Division of State and Private Forestry, Region 1; and PNW-2203.

14. Aerial photography and photo interpretation. Region 1, Division of Engineering and Division of State and Private Forestry.

2/ If Region 1 test sites prove unsuitable and alternative suitable test sites are located in Region 6, then Region 6 Timber Management personnel will have involvement in all aspects of this test.

15. Training and supervision of field crews. Region 1, Division of State and Private Forestry; PNW-2208; PNW-2203; and MEDC.

16. Analysis of data and reporting of results. Region 1, Division of State and Private Forestry; PNW-2208; PNW-2203; and MEDC.

In conjunction with the pilot control project, MEDC and Dugway Proving Grounds (Department of Defense) will be evaluating meteorological equipment. This will include placing additional spray deposit assessment cards. The equipment will not be tested on all plots. No supporting funds or personnel will be needed from the pilot control project for this satellite study.

ORGANIZATION

The organization chart for this project is shown in Figure 3.

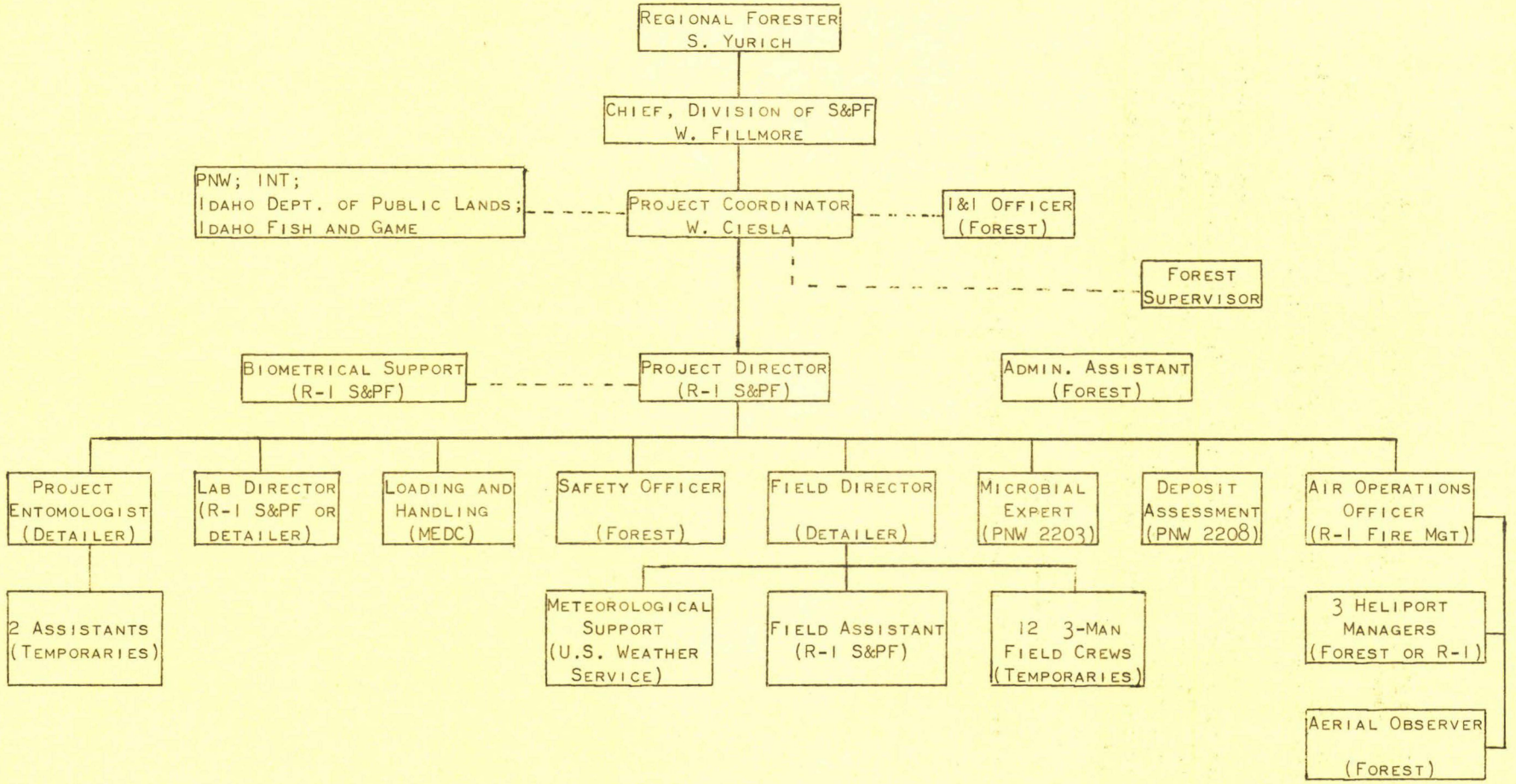


Figure 3.--Organizational chart for 1974 pilot control project for Douglas-fir tussock moth, Region 1. This organization will be duplicated at the Coeur d'Alene Mountain area and the Lookout Butte area.

FINANCIAL PLAN FOR 1974 PILOT CONTROL PROJECT FOR DOUGLAS-FIR TUSSOCK MOTH, REGION 1

Item	FY 1974	Rate	FY 1975	Rate
Materials				
Dipel	$ 80,000	20,000 lbs. @ $4/lb.		
Virus*	85,000	10,000 lbs. @ $8.50/lb.		
Carrier (molasses)	22,500	15,000 gal. @ $1.50/gal.		
Additives (dye, buffer, sunscreen)	12,000			
Aircraft**				
Helicopters	105,000	30,000 acres @ $3.50		
Chase planes	800			
Salaries				
Field crews	25,920	72 men @ $3/hr. for 15 days	$ 60,480	72 men @ $3/hr for 35 days
MEDC	11,880	9 men @ $88/day for 20 days		
Overtime	23,200		25,200	
Vehicle rental				
Pickup trucks	10,560	40 pickups @ $440/day for 24 days	18,040	40 pickups @ $440/day for 41 days
Gas and oil for vehicles	2,000		3,000	
3,000-gallon tankers	5,000	2 trucks @ $175/day for 14 days		
Per diem	8,000	16 men @ $20/day for 25 days	12,800	16 men @ $20/day for 40 days

*Financing of virus has already been covered; $44,000 worth of virus is now being contracted for, the remainder is on hand from a 1972 contract.

**May require some financing in 1975, depending on weather and larval development.

FINANCIAL PLAN FOR 1974 PILOT CONTROL PROJECT FOR DOUGLAS-FIR TUSSOCK MOTH, REGION 1, con.

Item	FY 1974	Rate	FY 1975	Rate
Equipment and supplies	$ 10,700		$ 1,800	
I&I	3,000			
Deposit assessments	3,500		8,100	
Freight				
Carrier	4,500			
Mixing unit	500			
Planning, training, and related activities	3,000			
Environmental monitoring	3,000		4,500	
Computer time			1,000	
Laboratory and coolers	400		600	
Aerial photography	1,500		1,500	
	$419,960		$137,980	

Total	$557,940
Virus costs	-85,000
Financing needed	$472,940

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UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE
Corvallis, Oregon 97331

APPENDIX I

REPLY TO: 4500 Forest Insect Research December 3, 1973

SUBJECT: Clean-up of nucleopolyhedrosis virus for the Douglas-fir tussock moth pilot test

TO: William M. Ciesla, Coordinator, Environmental Services
Division of State and Private Forestry

It is my understanding that you intend to use the tussock moth virus that is currently in stock at the PNW Station for the 1973 pilot control test. This poses another problem in addition to the mixing and application problems we discussed in Portland November 26 & 27. This virus stock is the "dirty" virus that I mentioned in Portland. We screened (filtered) the quantities of virus that were used for the 1972 field experiments to reduce the number of large extraneous particles (body parts and hairs). You will have to do the same for the ca. 7000-acre equivalents of virus you intend to use for the pilot test.

I have discussed this problem with Dr. Mauro Martignoni and Paul Iwal, who did the actual screening for the field experiment. We came up with the following screening procedure. The filtering process has to be done in a liquid suspension. It can't be done in a dry state.

A couple of days before a proposed application date, the virus, in the freeze-dried state, can be suspended in a 0.02 molar solution of Trizma-pH 7.1 buffer. Enough virus should be mixed to allow for about a 20% loss in polyhedra count during the filtration process. We suspended 10-acre equivalents ($1.0X10^{11}$ polyhedra) of virus in 150 ml of buffer, but we would recommend that you use a proportionately larger quantity of buffer since our suspensions were quite thick. Ten to 15 gallons of buffer per 1,000-acre equivalents would probably be a better ratio.

The Trizma-pH 7.1 buffer can be obtained from Sigma Chemical Company:

Sigma Chemical Co.
P.O. Box 14508
St. Louis, MO
Ph: 314-771-5750

You should specify reagent grade quality with an average molecular weight of 154.7. The pH should be checked, after the buffer solution has been mixed, but before the freeze-dried virus is added. The pH can be checked with a conventional portable pH meter. However, the

reading should not be taken immediately after putting the electrodes in the solution. One or two minutes should be allowed for the hydrogen ions to react with the probes. Triz buffer does not react well with pH paper, so a meter has to be used to check the pH.

A small capacity mixing tank, equipped with some type of recirculation, should be used for this process. The mixing equipment will have to be equipped with some type of filtering capability which allows for quick exchange and cleaning of the filtering screens. Three mesh sizes are recommended for the cleaning process; 50, 100, and 150 (maybe even a 200) mesh screens. The filtering process starts with the coarse mesh and progresses to the finer mesh screens as the larger hairs and body parts are strained from the virus suspension.

A polyhedra count will have to be made of the final screened virus suspension. Based on such a count, the proper amount of the screened virus suspension can then be added to the virus spray formulation to give dose of $1X10^{11}$ polyhedra per acre. Someone from Hank Thompson's project should work with someone from R-1, probably Mark McGregor, to familiarize them with the counting procedure and other bioassay details.

Further details of this clean-up problem and the requirements and design of the small mixing tank can be further discussed during the Missoula meeting, December 11, at which time we can get some input from MEDC. I feel that this whole clean-up process should be part of the study plan because "dirty" virus of this type might be encountered in the future, even though procurement contracts may specify the required quality.

If you have any questions, please feel free to give me a call.

JOHN NEISESS
Chemist

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE
3200 Jefferson Way
Corvallis, Oregon 97331

REPLY TO: 4500 Forest Insect Research December 14, 1973

SUBJECT: Cleaning of nucleopolyhedrosis virus preparation

TO: William Ciesla

I have some additional information concerning the cleaning of the virus.

1. The average weight (before screening) of the 1972-1973 nutrilite produced virus for the 10^{11} polyhedra/acre dose is 7.226 grams. A 1,000-acre dose would be 7.226 kilograms.

2. I was in error in the recommended ratio of virus to buffer solution. The correct ratio of virus to buffer is 1:10 (w/v). Thus, 72.26 liters of buffer solution (19.1 gallons) are needed for suspending 1,000-acre doses of virus prior to screening. Any ratio less than 1:10 (w/v) results in suspensions which are unsuitable for fast flow through the fine mesh screens. Therefore, the 10 to 15 gallons per 1,000-acre dose I suggested in the December 3 memo would not provide a sufficiently dilute suspension.

If you need any other information for the pilot test, either Milt Stelzer or I should be able to supply it. Sorry I wasn't able to be in Missoula for the meetings.

JOHN NEISESS
Chemist

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE

REPLY TO: 4500 Forest Insect Research January 17, 1974

SUBJECT: 1974 Microbial Pilot Test-formulations

TO: Tony Jasumback, MEDC

I am enclosing the information concerning the cleanup of the virus and technical information dealing with Santoquin - one of the stabilizers added to the CIB, as we discussed January 10, 1974. Dick and I were very happy to hear that you were going to be involved in the pilot project.

I got to thinking about the possible use of the large helicopters. If the larger aircraft are to be used, a larger nozzle tip size would be used than what we used with the Bell 47G. We have conducted tests with a 25% molasses-Dipel (B.t) formulation using T8010 tips and flying at 90 m.p.h. The resulting VMDs were 266 and 321 microns for 60 and 40 psi, respectively. We also used a molasses-Thuricide (B.t.) formulation which had about the same viscosity, but a lower surface tension as the Dipel formulation. The VMDs for this formulation were 199 and 231 microns at 60 and 40 psi. We did not use any screens in the application equipment. If a Bell 205 or 206 helicopter were used for the pilot tests the plugging problems that we encountered would essentially be eliminated by simply removing all the screens from the equipment. The formulations would still have to be thoroughly filtered or screened while mixing and loading.

We will be looking forward to hearing from you on your progress with the other mixing and handling problems.

JOHN NEISESS
Chemist

APPENDIX - Section B

THE DOUGLAS-FIR TUSSOCK MOTH IN THE NORTHERN REGION--A CARTOGRAPHIC HISTORY OF OUTBREAKS FROM 1928 TO 1973

by

Scott Tunnock, Entomologist
Environmental Services

ABSTRACT

The Douglas-fir tussock moth, *Orgyia pseudotsugata* McD., periodically defoliates Douglas-fir, true firs, and other host trees in forests of the western United States. In the Northern Region, these infestations occur about once every decade.

This history covers the earliest recorded outbreak in northeastern Washington from 1928 to 1930 and includes information about outbreaks in northern Idaho and eastern Washington from 1944 to 1947, northeastern Washington and northern Idaho from 1950 to 1955, northern Idaho and western Montana from 1961 to 1965, and the current outbreak in northern Idaho, northeastern Washington, and western Montana which began in 1970.

These outbreaks usually last from 2 to 4 years in forested areas and typically go through a buildup phase the first year, an outbreak phase the second year, and a declining phase the third year due to parasites and/or polyhedrosis virus. Exceptions are: It may take an outbreak 2 years to build up or occasionally natural control agents can cause a population collapse at the end of the second year.

INTRODUCTION

The Douglas-fir tussock moth, *Orgyia pseudotsugata* McD., is a defoliator of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, and true fir, *Abies* sp., forests in the western United States. Larvae (fig. 1) can feed on new foliage and older growth; consequently, heavy larval populations are capable of completely defoliating and killing trees in a single growing season (fig. 2). The tussock moth, then, is one of the most potentially destructive forest defoliators in the western United States.

This insect species was first recorded severely defoliating Douglas-fir near Chase, British Columbia, Canada, in 1918 (Evenden and Jost 1946). Since then, major epidemics have occurred every decade in the fir forests of western North America as Wickman's et al. (1973) history of epidemics in Oregon and California and this report illustrate.

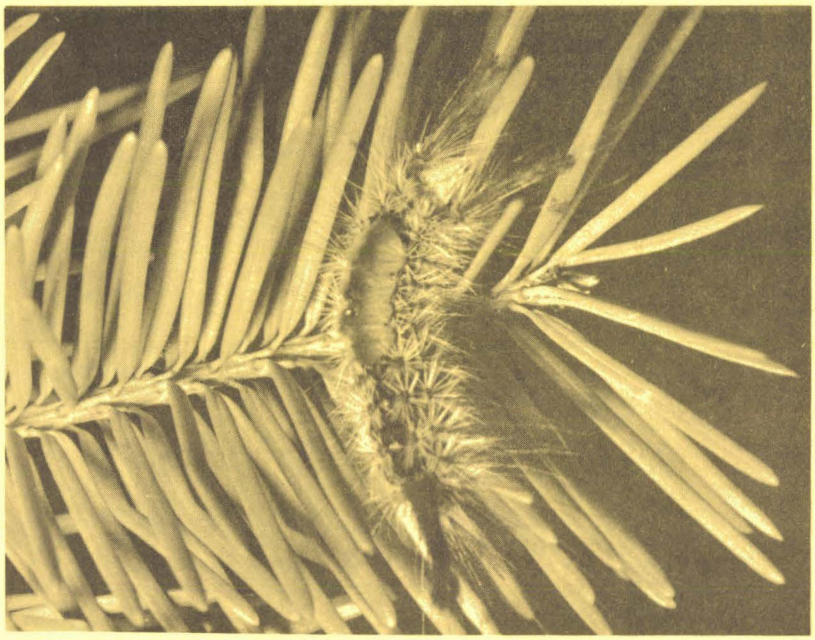

Figure 1.--Fully grown Douglas-fir tussock moth larva feeding on current needles of a fir tree.

Figure 2.--Mixed fir stand in northern Idaho heavily defoliated by the Douglas-fir tussock moth.

Recording the history of Douglas-fir tussock moth epidemics in the Northern Region at this time coincides with a severe epidemic in Oregon and Washington, and the beginning of one in northern Idaho which is a part of the Northern Region.

PAST OUTBREAKS

Northeastern Washington, 1928-1930.--Between 1928 and 1929, a severe epidemic of Douglas-fir tussock moth developed throughout forested areas near Northport, Washington. This epidemic reached its peak on the Colville National Forest, Washington, in 1930, and by then had destroyed 300 million board feet of commercial timber (Evenden and Jost, 1946).

Northern Idaho and eastern Washington, 1944-1947.--Spot infestations in ornamental (fig. 3) and shelterbelt trees around farms and residences appeared in 1944. These spots were located at Rathdrum, Troy, Moscow Mountain, and Sandpoint, Idaho, and near Palouse, Washington. Additional spot infestations were recorded in 1945 near Farragut, Coeur d'Alene, Rathdrum, Worley, and Geneese, Idaho. Two forested areas were defoliated that year; 320 acres were infested near Viola, Idaho, and 80 acres 35 miles northwest of Viola between Fairfield and Latah, Washington, were damaged (Evenden and Jost, 1946).

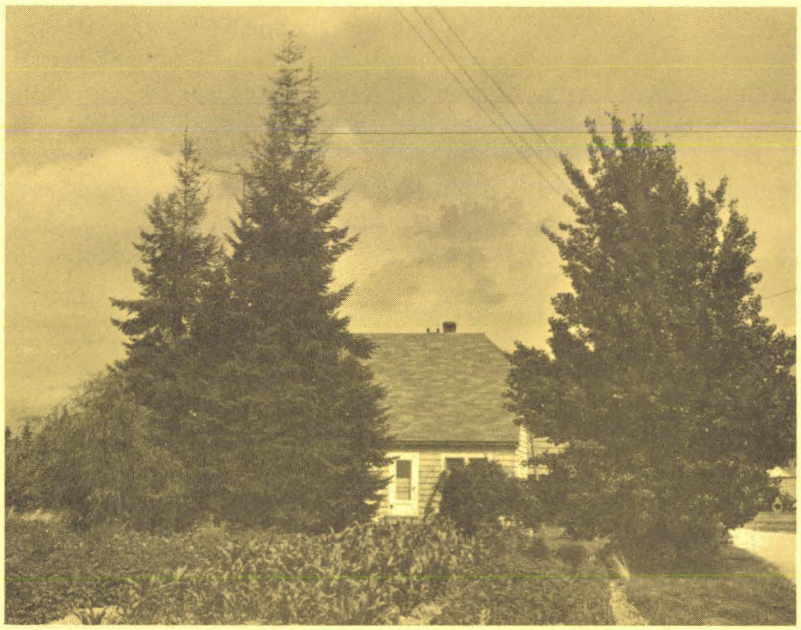

Figure 3.--Ornamental fir trees with tops defoliated by the Douglas-fir tussock moth.

An aerial reconnaissance of the St. Joe National Forest, Idaho, was made in 1946 and tussock moth defoliation was visible over 350,000 acres in Clearwater, Latah, and Benewah Counties in Idaho (fig. 4). Host type was Douglas-fir, grand fir, *Abies grandis* (Dougl.) Lindl., and Engelmann spruce, *Picea engelmannii* Parry. A ground survey showed approximately 447,000 acres were infested with variable population levels of tussock moth larvae. This acreage was broken down into the following four classifications (Evenden and Jost, 1946):

	Acres
1. Heavy defoliation - injury varies from 50 percent to practically complete elimination of all foliage	150,000
2. Medium defoliation - injury varies from a complete defoliation of 3 to 4 feet of extreme top of tree to 50 percent of total foliage	70,000
3. Light defoliation - visible damage to new growth on limb and extreme top of tree	130,000
4. Insects present within area but no injury visible	97,000

In 1947, more than 400,000 acres were sprayed from the air with 1 pound of DDT in 1 gallon fuel oil per acre to control the tussock moth in northern Idaho. Spraying began on May 22 and terminated on July 2. No living larvae were found anywhere in the treated areas. This great success was attributed to spraying larvae when they were most susceptible--the first three instars. Workers estimated that spraying prevented the almost certain defoliation of host trees containing 1½ billion board feet of merchantable sawtimber valued at $4,320,000, and host trees containing another 1.2 billion board feet immediately adjacent were saved from possible defoliation. In addition, 140,000 acres almost completely defoliated in 1946 were saved from being killed by additional defoliation in 1947 (Evenden and Jost, 1947).

Additional outbreaks were located during the summer of 1947 on 10,000 acres near Colville, Washington, and on some 20,000 acres east of Orofino, Idaho. A combination of polyhedrosis virus and parasites terminated both these infestations before moths could emerge in the late summer of 1947 (Johnson, 1949).

Northeastern Washington and northern Idaho, 1950-1955.--The Douglas-fir tussock moth was reported in scattered areas of Spokane, Pend Oreille, and Stevens Counties, Washington, in 1950 and 1954 (Gibson and Terrell, 1955).

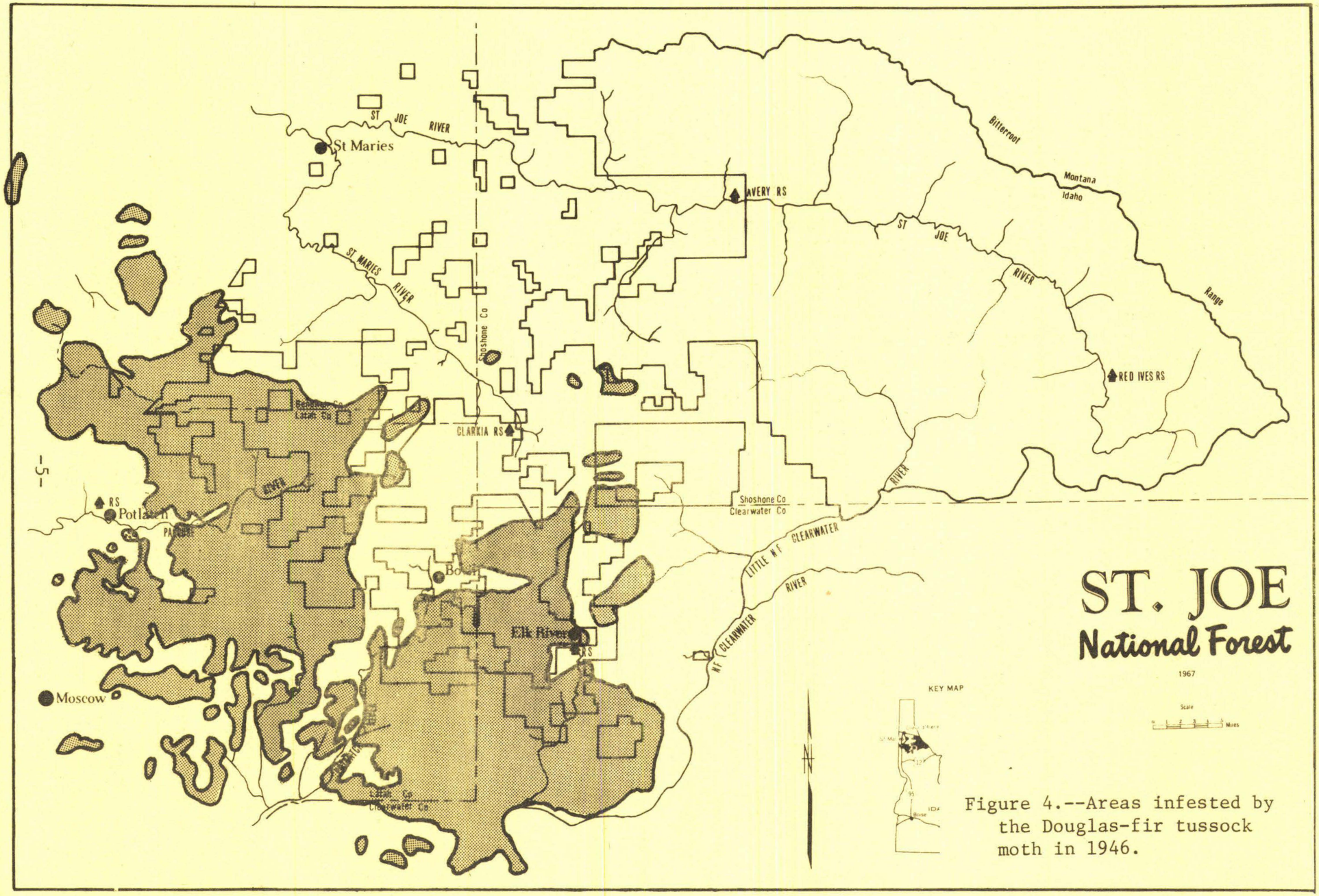

Figure 4.--Areas infested by the Douglas-fir tussock moth in 1946.

An aerial survey of these counties in 1955 showed that about 9,075 acres were defoliated (fig. 5). In Stevens County, 75 areas of from 1 to 2,000 acres were detected; 10 spots in farm yards were observed in Spokane County; and two farm yards were infested in Pend Oreille County (Terrell, 1955). Also, in 1955, 30,600 acres had various degrees of defoliation east of Orofino, Idaho. Studies of the Washington-Orofino infestations during the winter of 1955-56 showed that parasitism of moth cocoons in all forested areas averaged 71.3 percent in Washington and 78.0 percent near Orofino. All these infestations reached their peak in 1955 and by the end of that year subsided (Dodge, 1956).

Northern Idaho and western Montana, 1961-1965.--In 1961, Douglas-fir, grand fir, and ornamental spruce trees near farms and private homes were heavily defoliated in Moscow, Clark Fork, and Bonners Ferry, Idaho. Nearly 50 percent of the infested trees in and near Bonners Ferry, and about 90 percent in Moscow were sprayed by private landowners with various insecticides during the summer of 1961 (Tunnock, 1962). Aerial surveys of northern Idaho and northeastern Washington during 1962 detected infestation centers in or near Copeland, Moyie, Albeni Falls, Hayden Lake, Coeur d'Alene, St. Maries, Orofino, North Bonners Ferry, Idaho, and Colbert, Washington. Personnel of the Palouse Ranger District, St. Joe National Forest, Idaho, detected small numbers of larvae in some of their forested areas (Tunnock, 1963).

In 1963, surveys located four new infestations in addition to those described above. Six ornamental trees around a home near Algoma Lake south of Sandpoint, Idaho, contained current year egg masses. Forested areas near Mineral Mountain Lookout, north of Potlatch, Idaho, contained egg masses. About 1,400 acres of Douglas-fir southwest of Kalispell, Montana, were heavily defoliated for the first time. By the end of the summer a polyhedrosis virus killed most of the larvae in the northern portion of this infestation. Near Lion Lake, close to Hungry Horse Reservoir, Montana, about 10 acres of timbered land were infested. No 1963 egg masses were found, however (Tunnock, 1963).

There were indications that an epidemic was building up in northern Idaho when larvae were found on the Palouse District in 1962 and egg masses were present on Mineral Mountain in 1963. By 1964, defoliation visible from the air covered about 70,000 acres in Benewah and Latah Counties, Idaho. Ground surveys disclosed that the epidemic extended from Moscow to Coeur d'Alene Lake and covered a total of 191,000 acres. The number of acres defoliated near Kalispell, Montana, increased to 4,800, and about 250 acres of Douglas-fir were infested south of Polson, Montana. Several new spot infestations appeared in 1964 near Spirit Lake, Rathdrum, Hauser Lake, Mica, Idaho, and at Big Fork, Troy, Tally Lake, Bonita, and Missoula, Montana (Figs. 6, 7, and 8). Since 1961, tussock moth populations gradually subsided in the following Idaho towns due to spraying or natural causes: Moscow, Orofino, St. Maries, Algoma Lake, Albeni Falls, Coeur d'Alene, Moyie, and Clark Fork (Tunnock, 1964).

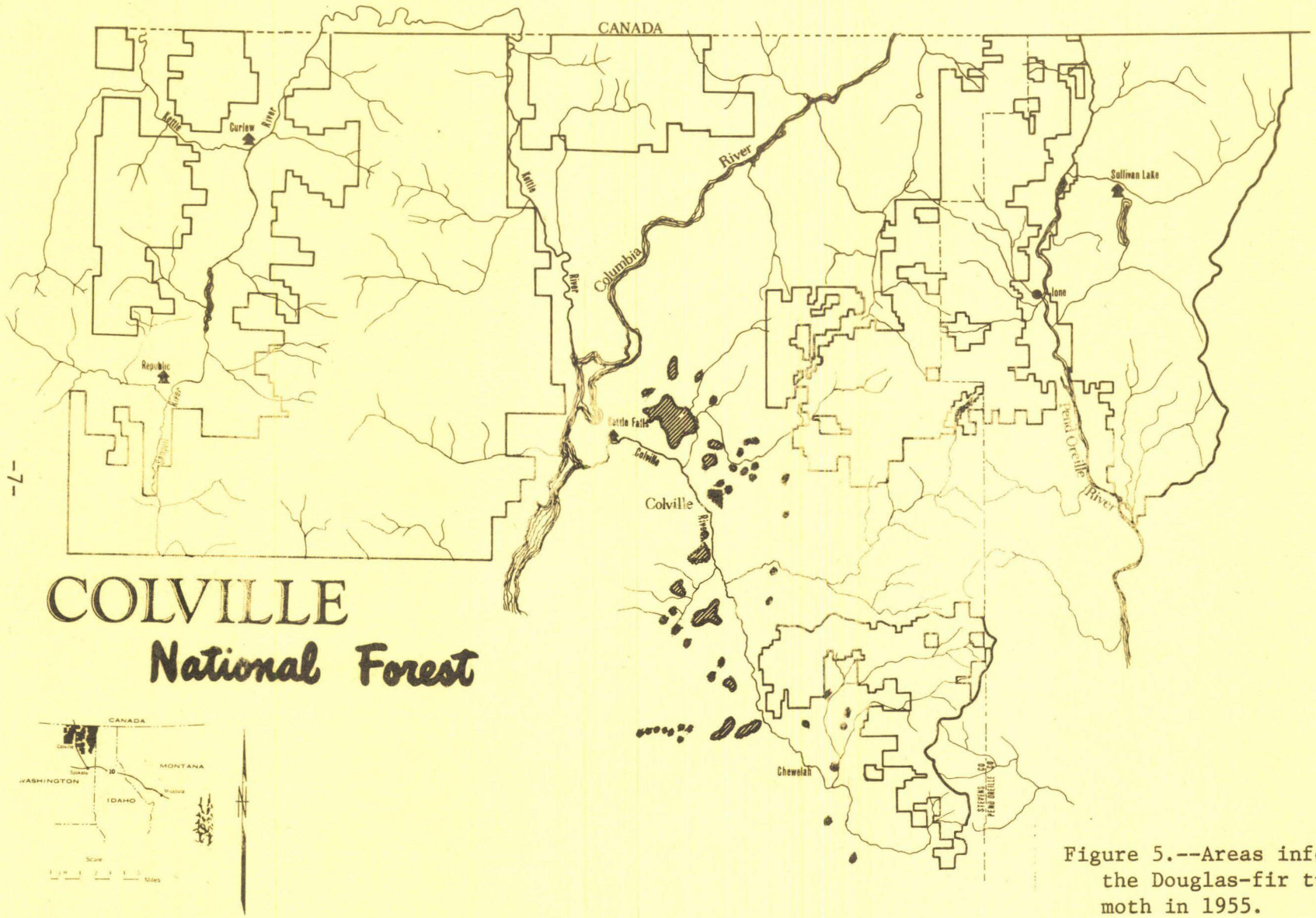

Figure 5.--Areas infested by the Douglas-fir tussock moth in 1955.

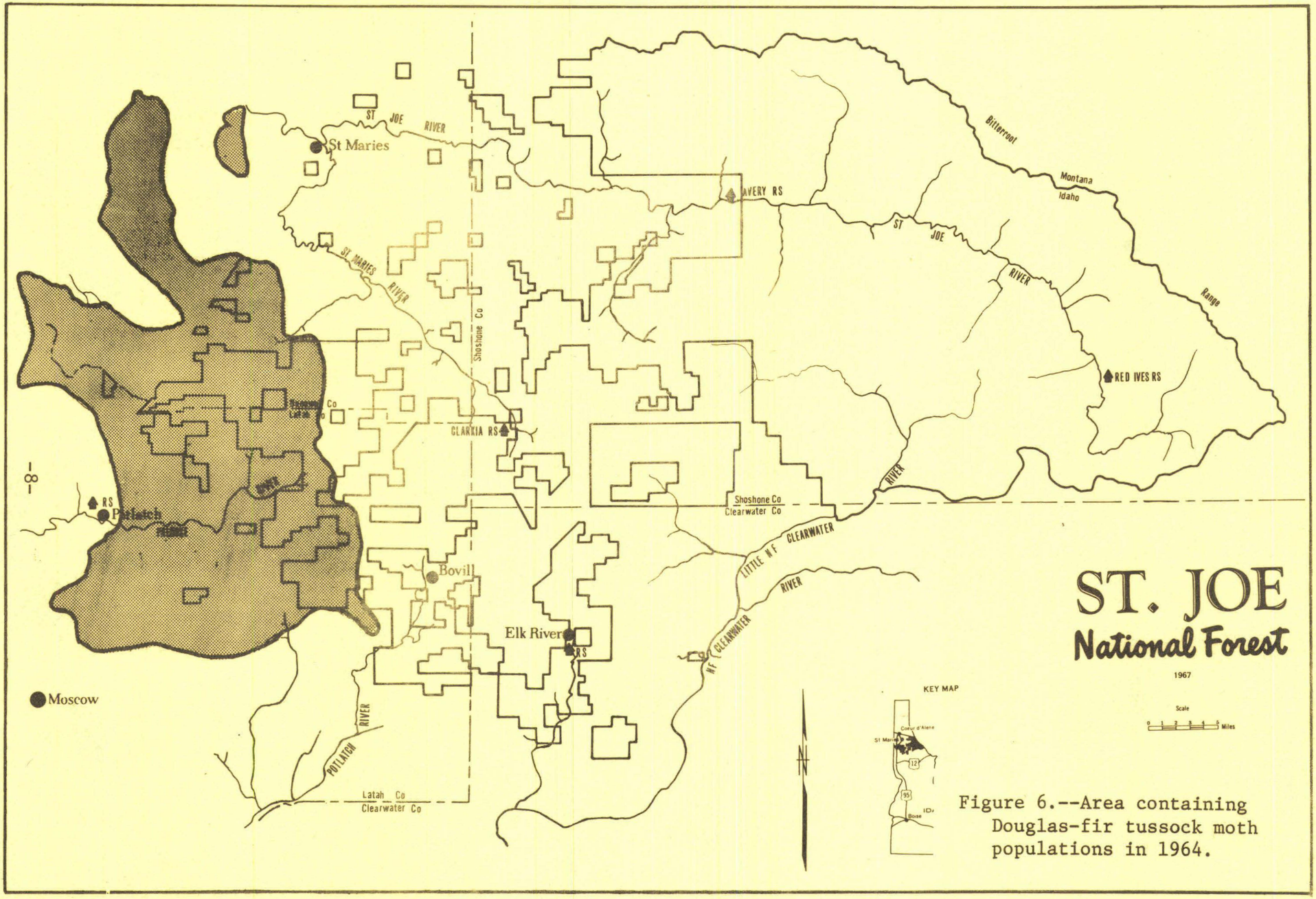

Figure 6.--Area containing Douglas-fir tussock moth populations in 1964.

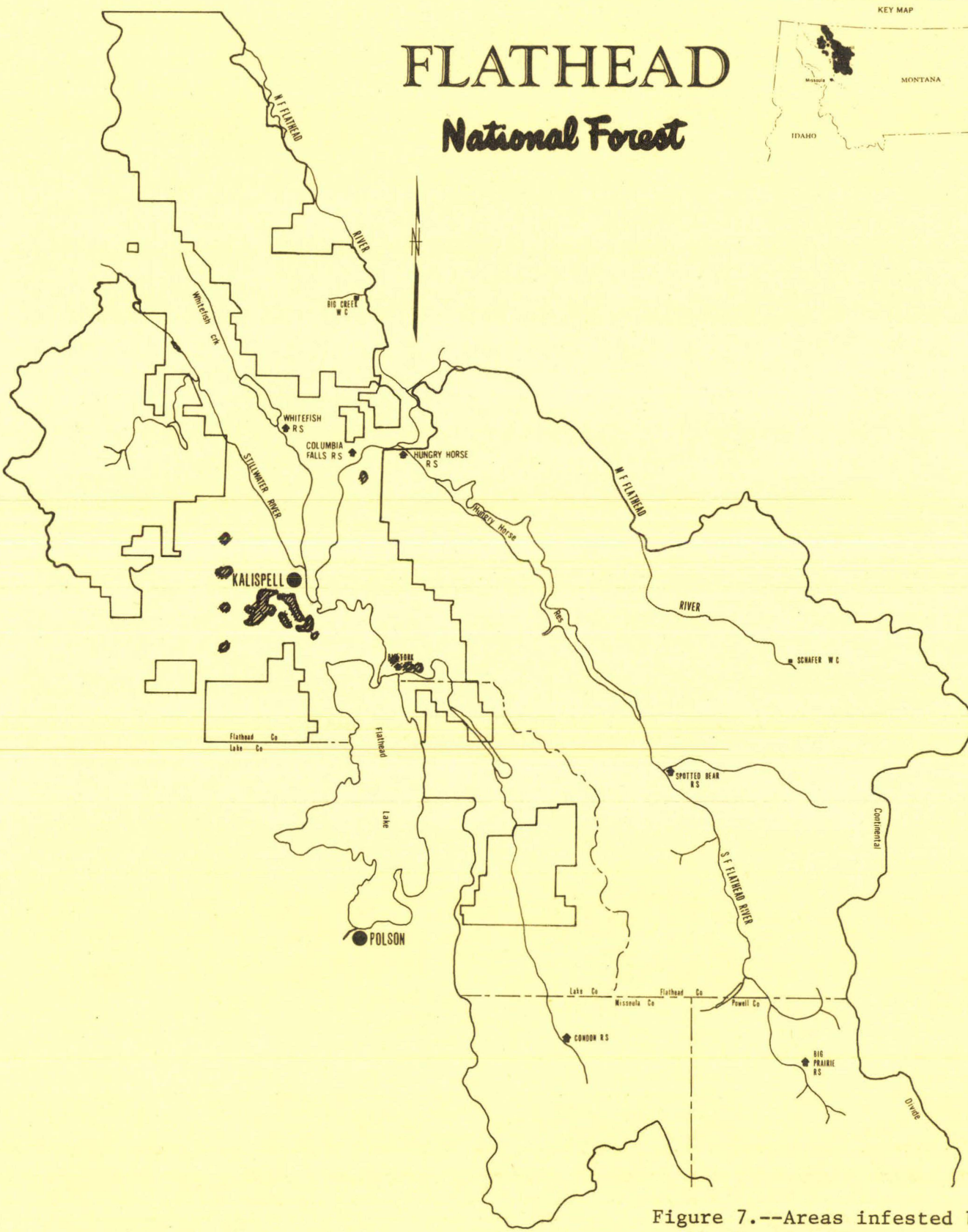

Figure 7.--Areas infested by the Douglas-fir tussock moth in 1964.

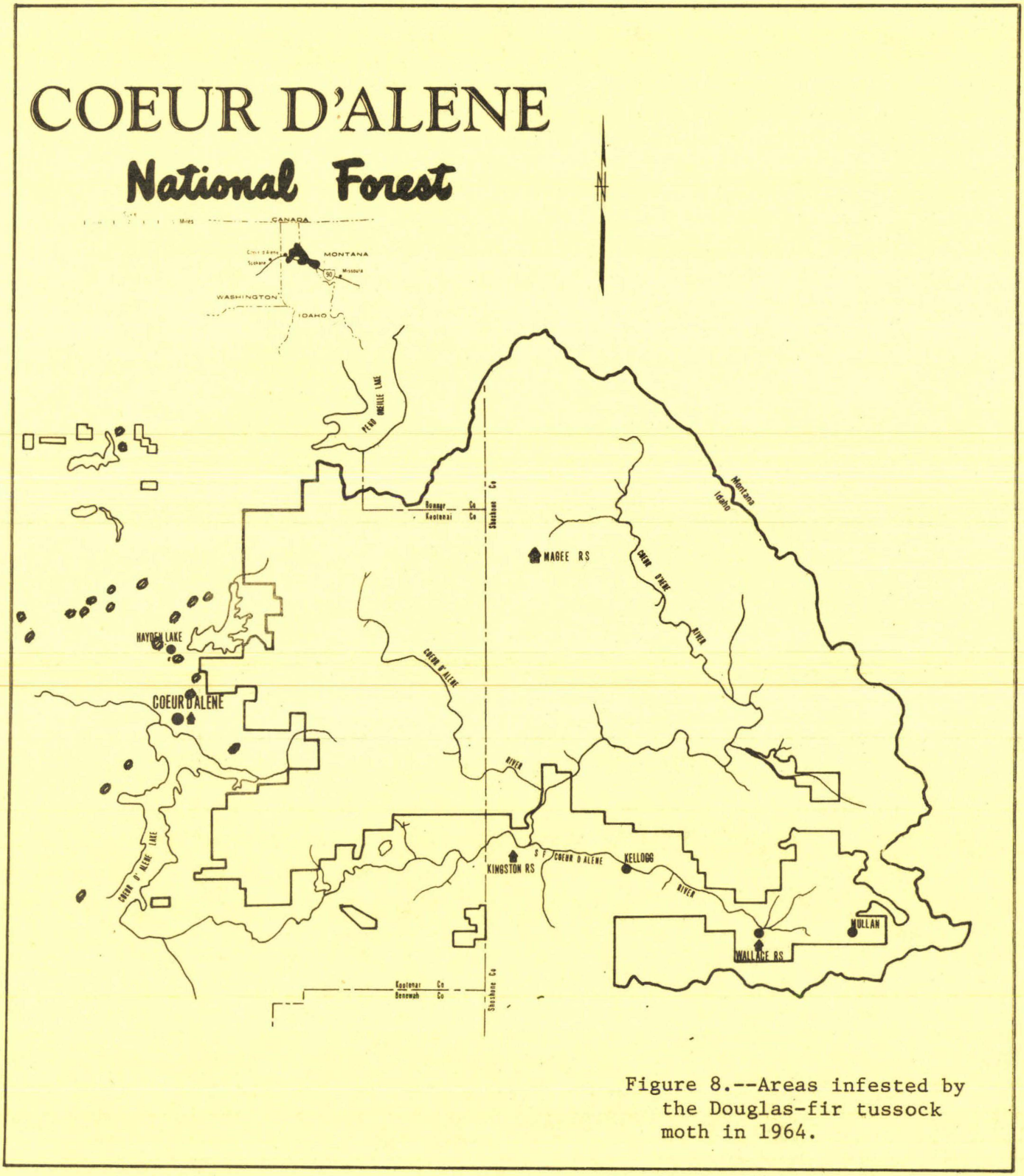

Figure 8.--Areas infested by the Douglas-fir tussock moth in 1964.

In 1965, tussock moth larvae caused various degrees of defoliation in only 50 of the 250 acres infested south of Polson, Montana, in 1964; in three new 10- to 40-acre areas south of Elmo, Montana; and within Benewah and Latah Counties, Idaho, the epidemic increased from 191,000 acres in 1964 to 225,000 acres in 1965 (figs. 9 and 10). A survey in October 1965 produced no current egg masses in Montana and only a few in Idaho. Observations showed that all the infestations had died out (Tunnock, 1965). Factors responsible for the collapse were nuclear polyhedrosis virus, parasites, and aerial applications of three-fourths pound of DDT per acre to about 120,000 acres in Idaho (Scribner, 1965). No infestations were found in 1966 in Montana or Idaho.

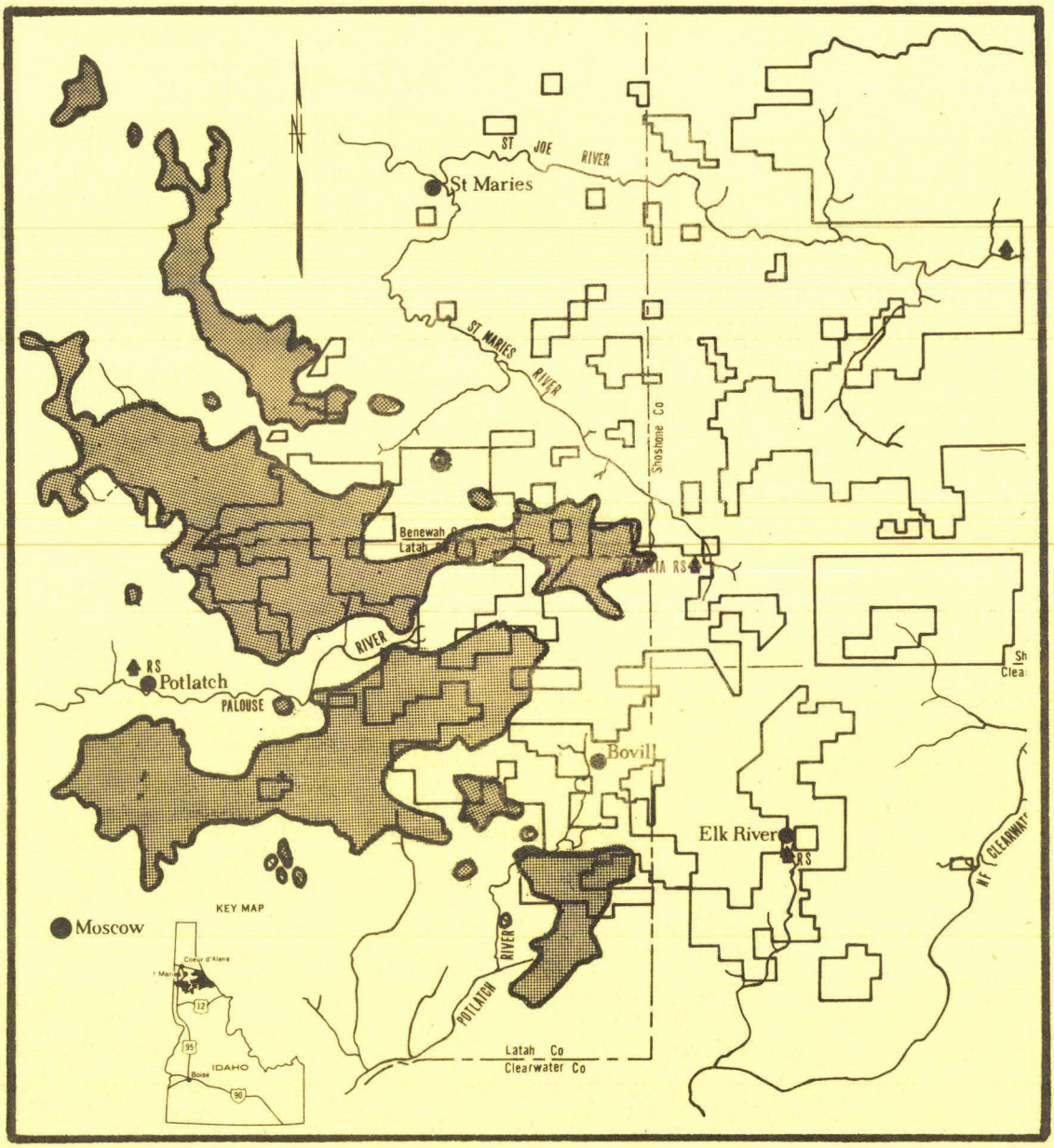

Figure 9.--Areas infested by the Douglas-fir tussock moth in 1965 on the St. Joe National Forest.

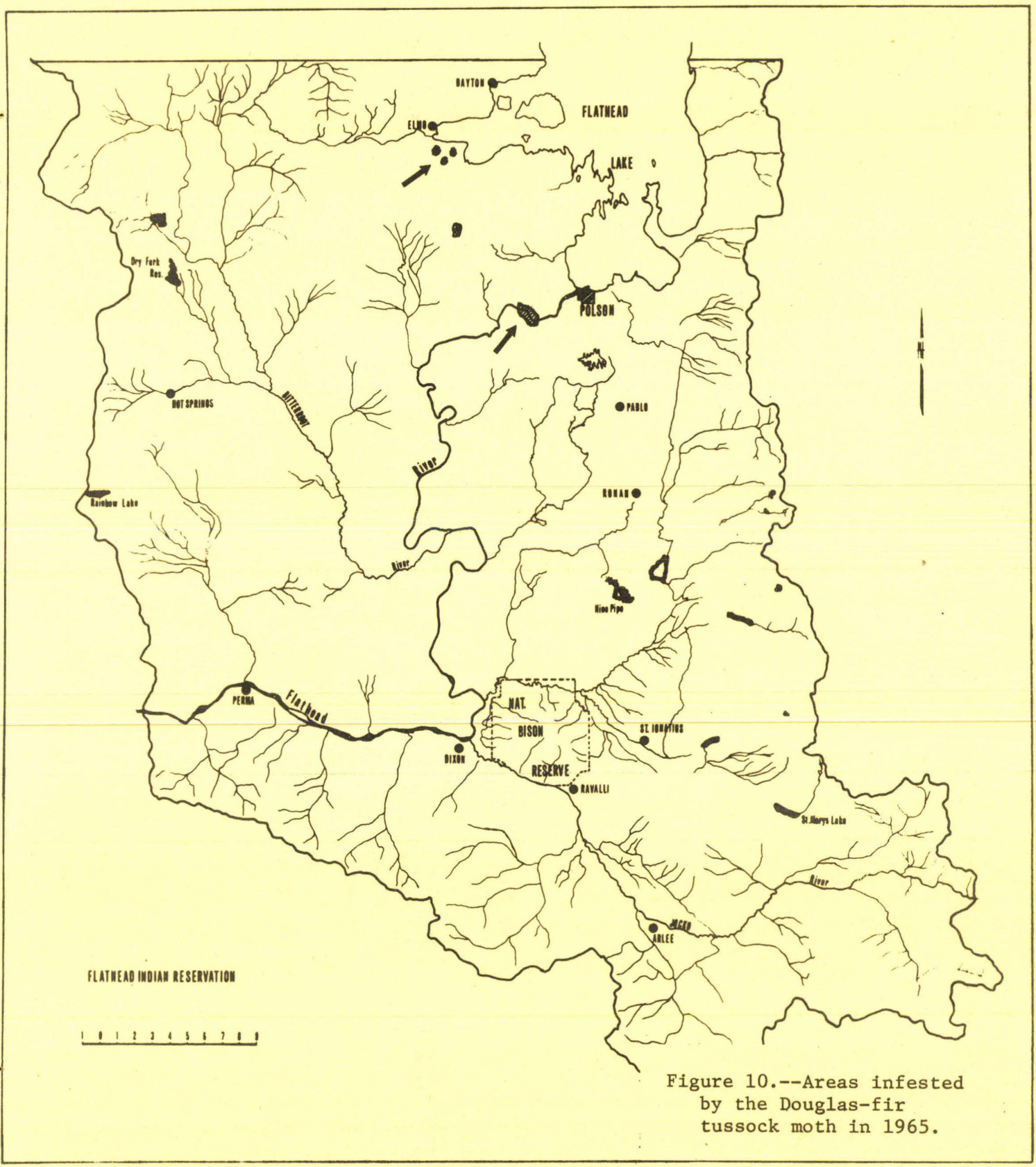

Figure 10.--Areas infested by the Douglas-fir tussock moth in 1965.

THE PRESENT OUTBREAK

Tussock moth populations were detected again in 1970 when several ornamental spruce were defoliated in Spokane, Washington, and Polson and Missoula, Montana. These infestations continued in 1971, and around several private homes in the Coeur d'Alene, Idaho, area grand fir, spruce, and Douglas-fir were defoliated (Tunnock and Honing, 1971).

In 1972, larvae were observed in individual trees in Spokane, Penix Canyon, and about 300 acres of defoliated Douglas-fir were sighted south of Kettle Falls, Washington, during the annual aerial insect and disease detection survey. Tussock moth reports in Idaho were scattered and confined mostly to ornamental trees. In Coeur d'Alene tussock moth larvae defoliated ornamental trees at 12 residential sites. Two ornamental spruce in a yard in Troy were defoliated, and damage was found along the Wolf Lodge Road, east of Coeur d'Alene, and in Post Falls and Moscow. Egg masses were numerous within a 100-acre logging area on Charles Butte, St. Joe National Forest, Idaho (Tunnock, 1972).

During February 1973 , a cooperative egg mass survey involving Idaho Department of Public Lands and the U.S. Forest Service was made in Benewah and Latah Counties in northern Idaho which have a history of past tussock moth outbreaks. It was felt that if egg masses were present on Charles Butte, they could be present in other areas. Twenty-eight sample plots were examined for evidence of tussock moth egg masses. Cocoons or egg masses were found in sample trees on 11 of the plots and in three other areas. Based on the data gathered in this survey, various degrees of defoliation were predicted for four general areas. Extensive defoliation was expected along the Palouse Range and south and southeast of Mica Mountain. Heavier defoliation was predicted from Mineral Mountain along Skyline Drive to McCroskey State Park. The largest area of defoliation was expected along the Divide from Windfall Pass south to the North-South Ski Area (Livingston and Tunnock, 1973).

An aerial survey during July and August 1973 to detect Douglas-fir tussock moth defoliation in the Northern Region showed damage did occur in the four areas within Benewah and Latah Counties, but was even more widespread than expected (table 1) (Tunnock, et al., 1973).

This aerial survey revealed that in the recorded history of Douglas-fir tussock moth epidemics in the Northern Region forested areas were infested for the first time on the Coeur d'Alene and Nezperce National Forests and on private lands south of Lewiston in Idaho and on the Lolo National Forest, Montana (fig. 11).

Table 1.--Defoliation by the Douglas-fir tussock moth in the Northern Region, 1973

Area	Acres
Washington	
Private lands along Columbia River	5,200
Idaho	
Coeur d'Alene National Forest	1,800
Adjoining State and private lands	80
St. Joe National Forest	20,000
Adjoining State and Private lands	50,000
Clearwater National Forest	0
Adjoining State and private lands	120
Private lands south of Lewiston	4,000
Nezperce National Forest	23,000
Montana	
Lolo National Forest	50
Adjoining State and private lands	300
Northern Region Total	104,550

In June 1973, the insecticide Sevin-4-oil (Carbaryl) was sprayed from a helicopter on three 60-acre plots in Benewah and Latah Counties, Idaho, to test its effectiveness against the Douglas-fir tussock moth. It was applied at the rate of 1 pound per acre in one-half gallon of carrier. This was a cooperative venture between the State of Idaho, Department of Public Lands; Union Carbide Corporation; and the U.S. Forest Service. The results showed only about an average of 56 percent population reduction could be attributed to the insecticide (Livingston, 1973)1/. However, significant amounts of foliage were saved when spray blocks were compared to untreated check blocks. Tussock moth population densities on these plots were quite low (50 to 80 insects per 1,000 square inches of foliage) in comparison to populations reported in other epidemics.

1/ Personal communication, Ladd Livingston, Idaho Department of Public Lands.

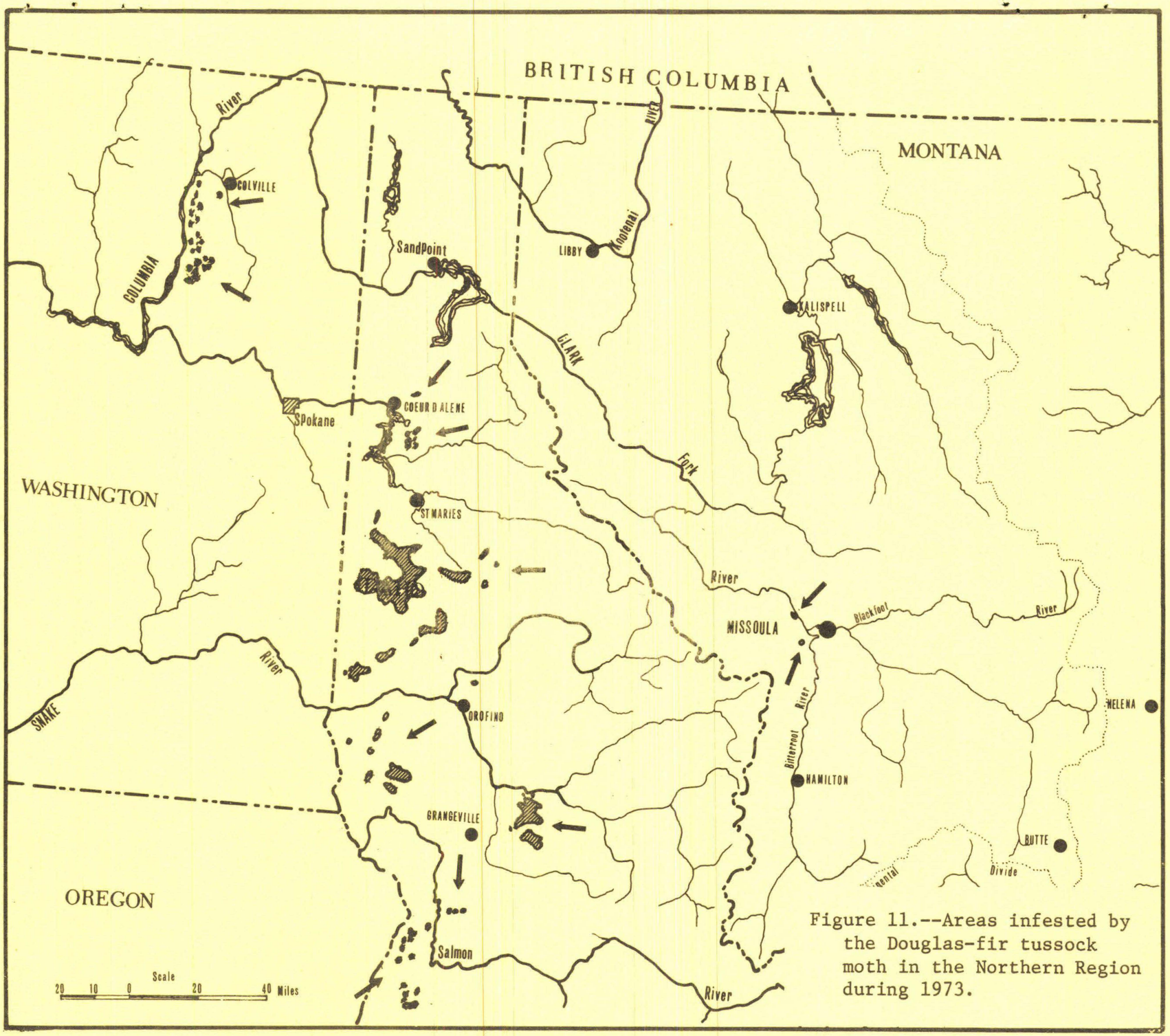

Figure 11.--Areas infested by the Douglas-fir tussock moth in the Northern Region during 1973.

DISCUSSION

Douglas-fir tussock moth outbreaks have followed a cyclic pattern in the forests of the Northern Region. Defoliation in forested areas is usually preceded by 1 to 2 years' of defoliation of individual ornamental trees in urban areas. Outbreaks in forested areas continue from 2 to 4 years in the Northern Region. A typical outbreak goes through a buildup phase the first year, an outbreak phase the second year, and a declining phase the third year due to parasitism and/or polyhedrosis virus. However, it may take an outbreak 2 years to build up, such as the 1961-65 outbreak in northern Idaho, or natural control agents may wipe out a population at the end of the second year, which happened during the 1954-55 outbreak in northern Idaho.

Three of the five recorded outbreaks in the Northern Region include commercial forest lands in Benewah and Latah Counties, Idaho. These are the 1944-47, 1961-65, and 1973- outbreaks. Defoliation maps prepared by survey personnel indicate that the same general areas were infested each time (figs. 4, 9, and 11) indicating that this region may be a warning zone for predicting Douglas-fir tussock moth epidemics. For some unknown reason, populations are released there first. This knowledge was helpful in locating egg mass sample plots for predicting the 1973 outbreak.

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POTENTIAL DOUGLAS-FIR TUSSOCK MOTH DAMAGE IN NORTHERN IDAHO IN 1974 BASED ON A 1973 FALL EGG MASS SURVEY

by

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ABSTRACT

An epidemic of Douglas-fir tussock moth was detected in northern Idaho in 1972. In 1973, aerial surveys showed that nearly 100,000 acres contained various degrees of visible defoliation. An egg mass survey of five reporting units made in the fall of 1973 determined potential for damage in 1974. Based on new egg mass densities and new to old egg mass ratios, damage is predicted to be sufficiently high to warrant control on 34,138 acres in the Coeur d'Alene unit, 64,779 acres in the St. Joe unit, 4,433 acres in the Clearwater unit, 4,762 acres in the Craigmont unit, and 33,501 acres in the Nezperce unit. Total acres qualifying for treatment are 141,613. In addition, some damage might occur on 81,554 acres within the five units. These areas will be further evaluated to determine if they qualify for treatment.

INTRODUCTION

Outbreaks of the Douglas-fir tussock moth, *Orgyia pseudotsugata* McD., have occurred in northern Idaho on a cyclic basis. History of these outbreaks has been reviewed by Tunnock (1973).

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Indications that the current outbreak was building up were discovered in Coeur d'Alene, Idaho, during 1971 when several ornamental firs were defoliated (Tunnock and Honing, 1971) and in 1972 when egg masses were found within a 100-acre logging unit near Charles Butte, St. Joe National Forest, Idaho (Tunnock, 1972). A February 1973 egg mass survey conducted in areas of previous outbreaks substantiated the buildup and predicted a minimum of 50,000 acres of visible defoliation (Livingston and Tunnock, 1973). Aerial surveys completed during the summer of 1973 showed nearly 100,000 acres with varying degrees of visible defoliation (Tunnock, et al., 1973).

The tussock moth is an important defoliator of true firs, *Abies* spp. L., and Douglas-fir, *Pseudotsuga menziesii* var. *glauca* (Beissner) Franco, over much of western North America. In Idaho, the favorite hosts are Douglas-fir and grand fir, *Abies grandis* (Dougl.) Lindl. However, if preferred food is scarce, the tussock moth will feed on any coniferous species.

Damage to trees is caused by the newly hatched larvae feeding on new foliage, causing it to shrivel and turn brown. By mid-July, larger larvae feed on both new and old foliage, first stripping the tops of trees and outermost portions of branches, then feeding into the inner crown (Mason and Baxter, 1970; Wickman, et al., 1971). While many trees are only top killed, occasionally they may be completely defoliated and killed in one season (Tunnock 1964). Trees weakened through defoliation have been reported to suffer pronounced growth loss, and many are subsequently attacked by bark beetles (Wickman, 1963).

To predict what this outbreak would do in 1974, a broad-scale egg mass survey was conducted during October and November 1973. The survey was a cooperative effort of a special task force established by the Northern Rockies Forest Pest Action Council. Participants included the State of Idaho Department of Public Lands; U.S. Forest Service, Region 1; Potlatch Forests, Inc.; Bureau of Indian Affairs; Corps of Engineers; and University of Idaho.

The following were the objectives of the egg mass survey:

1. Determine potential 1974 tussock moth population levels in and adjacent to the aerially visible defoliated areas.

2. Determine extent of infested area outside aerially visible defoliated areas.

3. Predict potential defoliation for 1974.

4. Classify areas where applied control may be advisable.

5. Estimate impact of natural factors (parasites, predators, disease, etc.) on the 1974 tussock moth population.

This report describes results of an egg mass survey, which provides, in part, the biological basis for recommendations for or against applied control in the various infested areas of northern Idaho. Additional observations will be made to estimate levels of natural virus incidence and other factors which might influence 1974 population levels. These will be reported at a later date.

METHODS

The zone of infestation was divided into five survey units. These units were designated Coeur d'Alene, St. Joe, Clearwater, Craigmont, and Nezperce.

Plots to be sampled were tentatively marked on maps to gather information within and around all known defoliated areas and within adjacent areas where defoliation had occurred in past outbreaks. Plots were established on the basis of access and availability of host type. Where access was available, every section within the defoliated areas was sampled. Helicopters were used in some of the roadless areas of the Nezperce unit.

Eight trees were sampled in each plot. A sample plot consisted of four limbs cut from the midcrown of each of eight trees. The extreme length and width of the foliar area of each branch, the number of new and old egg masses, and the presence or absence of cocoons on each branch were recorded. Five new egg masses were collected from each plot when new egg masses were found. These eggs are to be hatched in February to determine incidence of nucleopolyhedrosis virus on the plot. Also, the number of viable eggs per thousand square inches of foliage will be determined from new egg masses on each plot.

Survey data was utilized to compute number of new and old egg masses per thousand square inches of foliage, the ratio of old to new egg masses, and the abundance of cocoons for each plot. Resultant data was analyzed by a key developed by the working group of the Interagency Douglas-fir Tussock Moth Steering Committee to determine areas qualifying for control at this time.

1. No survey data collected in the area[3/] (6)

1a. Survey data collected in the area (2)

2. No new egg masses on plots - low risk[4/]

2a. New egg masses on plots (3)

3/ Usually a land section of approximately 640 acres in size, but can be smaller if necessary in order to determine treatment needs more precisely.

4/ Low risk = control not necessary for these areas.

3.	Egg mass density equal to or greater than 0.1 per thousand square inches of foliage - high risk[5/]	(7)
3a.	Egg mass density less than 0.1 per thousand square inches of foliage	(4)
4.	New to old egg mass ratio 1:1 or greater - high risk	(7)
4a.	New to old egg mass ratio less than 1:1	(5)
5.	Average egg mass count on time plots 15 or more - high risk	(7)
5a.	Average egg mass count on time plots less than 15 - low risk	
6.	Unsampled areas (sections) adjacent to one or more areas (sections) which equal or exceed the high risk criteria above - high risk	(11)
6a.	Unsampled areas (sections) not adjacent to one or more areas (sections) which equal or exceed the high risk criteria above - low risk	
7.	Virus level equal or greater than 50 percent - low risk	
7a.	Virus level less than 50 percent	(8)
8.	Area (plot) located in defoliation class I or II - high risk	(10)
8a.	Area (plot)located in defoliation class II or IV	(9)
9.	Virus level equal or greater than 30 percent - low risk	
9a.	Virus level less than 30 percent - high risk	(10)

5/ High risk = control recommended for these areas.

10. Viable egg density in sampled areas less than 20 eggs per 1,000 square inches foliage - low risk

10a. Viable egg density on sampled areas equal or greater than 20 eggs per 1,000 square inches foliage - high risk.

11. Unsampled area adjacent to an area meeting the high risk criteria after the virus and viable egg levels have been determined - high risk

11a. Unsampled area not adjacent to an area meeting the high risk criteria after the virus and viable egg levels have been determined - low risk.

RESULTS

A total of 400 plots were established within the five units. Table 1 summarizes data (Appendix Table 1) from all plots which contained new egg masses. New and old egg masses per thousand square inches of foliage and old to new egg mass ratios are listed.

Table 1.--Summary of data from plots with new Douglas-fir tussock moth egg masses

Unit	Number of plots containing new eggs	Range of egg masses per 1,000 sq. in. foliage		Range of old to new egg mass ratios
		New	Old	
Coeur d'Alene	28	.01 to .73	0 to .12	1:0.3 to 1:35
St. Joe	64	.01 to 3.6	0 to 1.5	1:0.2 to 1:22
Clearwater	5	.05 to .10	0 to 2.5	1:0.07 to 1:2
Craigmont	4	.02 to .31	.04 to .29	1:0.5 to 1:1.2
Nezperce	31	.02 to 5.3	0 to 1.3	1:0.03 to 1:7
Total	132			

Table 2 summarizes data (Appendix Table 2) taken from all plots which did not contain new egg masses. The presence of cocoons only, old egg masses, or no evidence of tussock moth activity are listed.

Table 2.--Summary of data from plots which did not contain new Douglas-fir tussock moth egg masses

Unit	Number of plots with no new eggs	Number of plots containing		
		Cocoons only	Old egg masses	No cocoons or eggs
Coeur d'Alene	45	24	6	15
St. Joe	147	77	21	49
Clearwater	27	11	9	7
Craigmont	16	11	3	2
Nezperce	33	15	5	13
Total	268	138	44	86

Table 3 shows the results of the surveys on the five units by presenting the number of acres with possible tussock moth activity, acres of recommended control, and acres that might need to be sprayed after further evaluation. Narrative descriptions of each unit follow table 3.

Table 3.--Acres of possible activity, proposed treatment, and treatment option in northern Idaho during 1974

Unit	Possible activity	Proposed treatment	Treatment option
Coeur d'Alene			
State and private		4,172	2,008
Forest Service		29,966	12,726
Subtotal	55,081	34,138	14,734
St. Joe			
Coeur d'Alene IR		4,500	0
State and private		45,352	36,681
Forest Service		14,927	5,615
Subtotal	234,549	64,779	42,296
Clearwater			
State and private		2,686	861
Forest Service		1,747	547
Subtotal	81,552	4,433	1,408
Craigmont			
State and private		4,762	7,162
Forest Service		0	0
Subtotal	21,338	4,762	7,162

Table 3.--Acres of possible activity, proposed treatment, and treatment option in northern Idaho during 1974, con.

Unit	Possible activity	Proposed treatment	Treatment option
Nezperce			
State and private		1,312	1,045
Forest Service		32,501	14,909
Subtotal	112,301	33,501	15,954
North Idaho total	504,821	141,613	81,554

Coeur d'Alene unit.--The Coeur d'Alene unit consists of an area bordered on the south and east by the Coeur d'Alene River, by Coeur d'Alene Lake on the west, and extending north across Interstate 90 into the headwaters of the Skitwish, Marie, Stoney, and Curran Creek drainages.

An isolated population site due east of Dalton Gardens, Idaho, on the ridge containing East and West Canfield Buttes was also sampled.

Aerial detection surveys during 1973 found 1,880 acres with visible defoliation due to the tussock moth. Fall egg mass surveys indicated some activity (cocoons, larvae, or old egg masses) on a total of 55,081 acres. Of this, 34,138 acres (fig. 1) have a population that warrants control, with 14,734 additional acres that may need treatment, pending further investigation.

This area did not show any defoliation in 1972 and appears to contain a building population. There is a distinct possibility that this unit could increase in size during 1974 as survey crews found tussock moth cocoons in most plots on the northern edge of the area. The infestation may extend farther north than could be sampled at this time.

St. Joe unit.--This area is the largest unit within the current Idaho infestation. It is roughly bordered on the north by the St. Joe River, on the west by the Idaho-Washington border, on the south by Idaho Highway 81, leading from Moscow to Elk River and extending to the east as far as Range 3 east, Boise Meridian.

Aerial detection surveys conducted in 1973 located a total of 70,000 acres with varying degrees of defoliation. No defoliation was observed in 1972. Subsequent monitoring has shown the Douglas-fir tussock moth might be active on approximately 234,549 acres. Fall egg mass surveys have shown that there is a high risk for damage on 64,779 acres (fig. 2) with populations being high enough to recommend control. In addition,

Fig. 1

DOUGLAS-FIR TUSSOCK MOTH EGG MASS SURVEY ON COEUR D'ALENE UNIT - 1973

Spray option - more evaluation needed 1974

Proposed control area - 1974

R5W
R4W
R3W
R2W
R1W
R1E
R2E
R3E
46N
T45N
T44N
T43N
T42N
T41N
T40N
FIG.2
DFTM EGG MASS SURVEY ON ST. JOE UNIT-1973
SPRAY OPTION-MORE EVALUATION NEEDED-1974
PROPOSED CONTROL AREA-1974

there are 42,296 acres with populations that may warrant control pending further investigation.

The Douglas-fir tussock moth population in this unit is in numerous widely scattered epicenters. The largest population centers are in a chain between Plummer and Moscow. These contain the majority of the areas where control is recommended. This is the same basic area where outbreaks of the tussock moth developed in 1945 and 1964.

Clearwater unit.--Only 120 acres were defoliated in this unit which is in Clearwater County between the North Fork of the Clearwater River and the Middle Fork to the south.

Egg mass counts classified 4,433 acres (fig. 3) as high risk for defoliation in 1974 and control is recommended. There is potential for defoliation of an additional 1,408 acres which may also need treatment in 1974. There was evidence of tussock moth populations in 81,552 acres within this unit.

Craigmont unit.--This infestation lies in the area between the Salmon and Clearwater Rivers. The known population is in an irregular band surrounding the Soldiers Meadows Reservoir area southwest of Winchester and southeast of Lewiston.

Aerial surveys during 1973 showed 4,000 acres with visible defoliation. Subsequent egg mass surveys and ground reconnaissance have shown some tussock moth activity in a total of 21,338 acres. Of this total 4,762 acres (fig. 4) have populations that warrant control, with an additional 7,162 acres that may need treatment pending further investigation.

Immediately to the south of this unit there is an extensive infestation of the western spruce budworm, *Choristoneura occidentalis* Freeman. The defoliation caused by the budworm may be masking additional Douglas-fir tussock moth populations similar to that in the Nezperce unit.

This is the first time that Douglas-fir tussock moth has been found in this area.

Nezperce National Forest unit.--This unit lies in Idaho County between the Middle Fork of the Clearwater River to the north and the Salmon River to the south. Defoliation occurring in 1973 was visible from the air on 23,000 acres mainly on the Selway and Salmon River Ranger Districts. Damage was heaviest on the Salmon District and some tree killing occurred in pure stands of Douglas-fir from 10 to 50 acres in size.

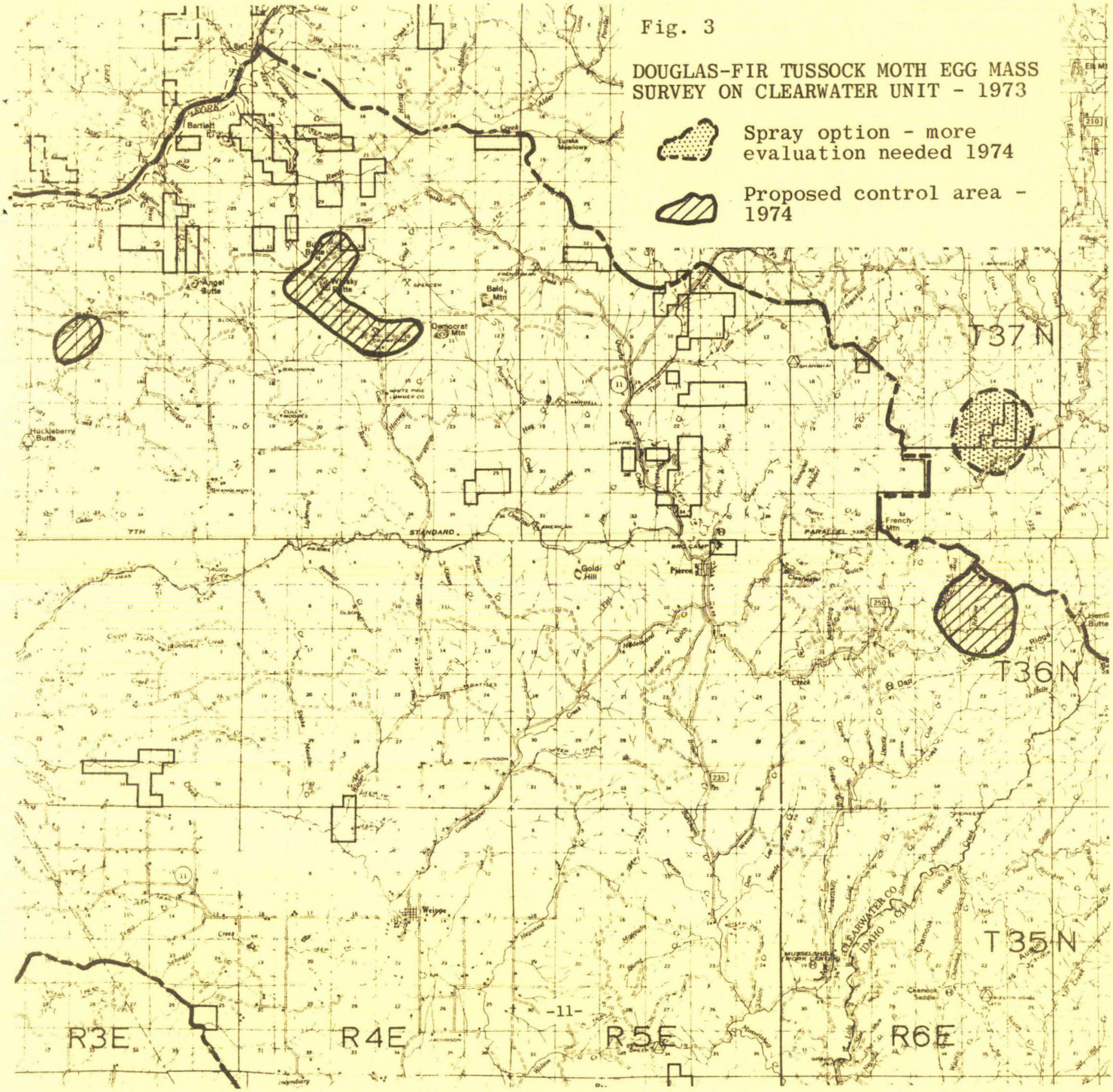
On the Salmon River District, egg mass counts classified 11,587 acres (fig. 5) as high risk and control is recommended for these areas. In addition, further sampling may indicate another 6,915 acres will need treatment. Altogether, there was possible tussock moth activity within 33,796 acres on this District.

Fig. 3

DOUGLAS-FIR TUSSOCK MOTH EGG MASS SURVEY ON CLEARWATER UNIT - 1973

Spray option - more evaluation needed 1974

Proposed control area - 1974



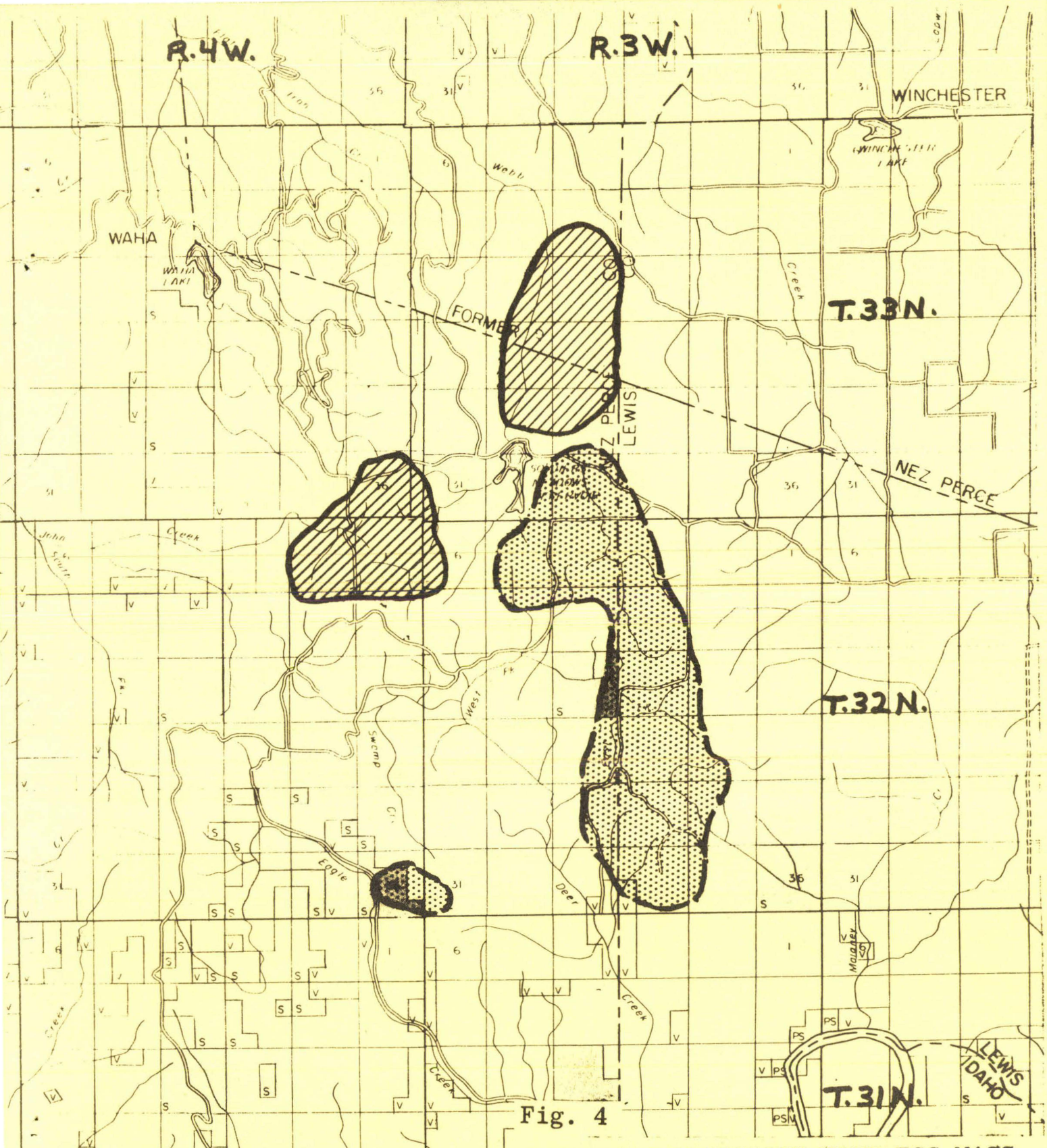


Fig. 4

DOUGLAS-FIR TUSSOCK MOTH EGG MASS SURVEY ON CRAIGMOUNT UNIT - 1973

Spray option - more evaluation needed 1974

Proposed control area - 1974

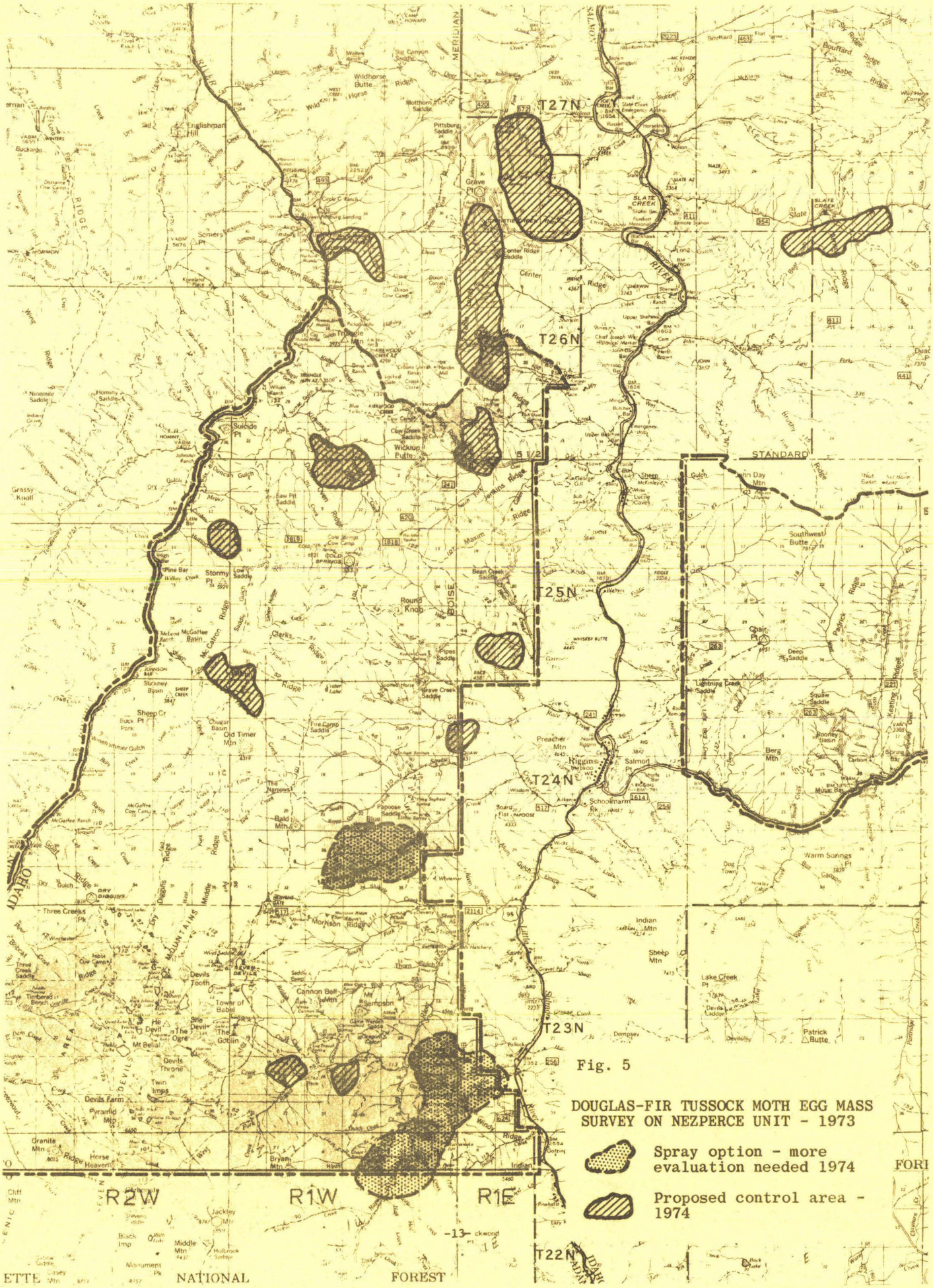


Fig. 5

DOUGLAS-FIR TUSSOCK MOTH EGG MASS SURVEY ON NEZPERCE UNIT - 1973

Spray option - more evaluation needed 1974

Proposed control area - 1974

ETTE NATIONAL FOREST

On the Selway District 21,914 acres (fig. 6) need treatment with an additional 9,039 acres of potential control area. Tussock moth infestation may occur over a total of 78,505 acres.

Totals for the Nezperce unit are:

Total area of possible activity	116,301 acres
Proposed spray area	33,501 acres
Spray potential area	15,954 acres

DISCUSSION

Within the five units there are numerous areas presently not included within the proposed or potential spray blocks that are thought to harbor tussock moth populations capable of causing unacceptable damage. Generally they are adjacent to existing proposed spray blocks. These areas were not sampled due to lack of access or suitable sample trees. Control decisions for these areas will be based on further evaluations including an aerial survey made after egg hatch and just prior to the intended treatment date.

During February 1974, eggs from new egg masses collected from many of the plots will be hatched in the laboratory to determine virus incidence. The key on pages 3, 4, and 5 will be used to decide whether the percentage of virus on a plot makes it high or low risk. If it falls into the low risk category, the area will be subtracted from the proposed spray acreage. Viable egg density on the plots will also be considered at this time. Additional areas that may be removed from consideration for chemical treatment include portions of the Coeur d' Alene and Nezperce units.

If the percentage of natural virus is sufficiently low within these two areas, they will be selected as sites for testing the nucleopolyhedrosis virus and the bactierium, *Bacillus thuringiensis*. The virus will be applied at the rate of 100 billion polyhedra in 2 gallons of water per acre, and the bacteria will be applied at the rate of 1 pound in 2 gallons of water per acre.

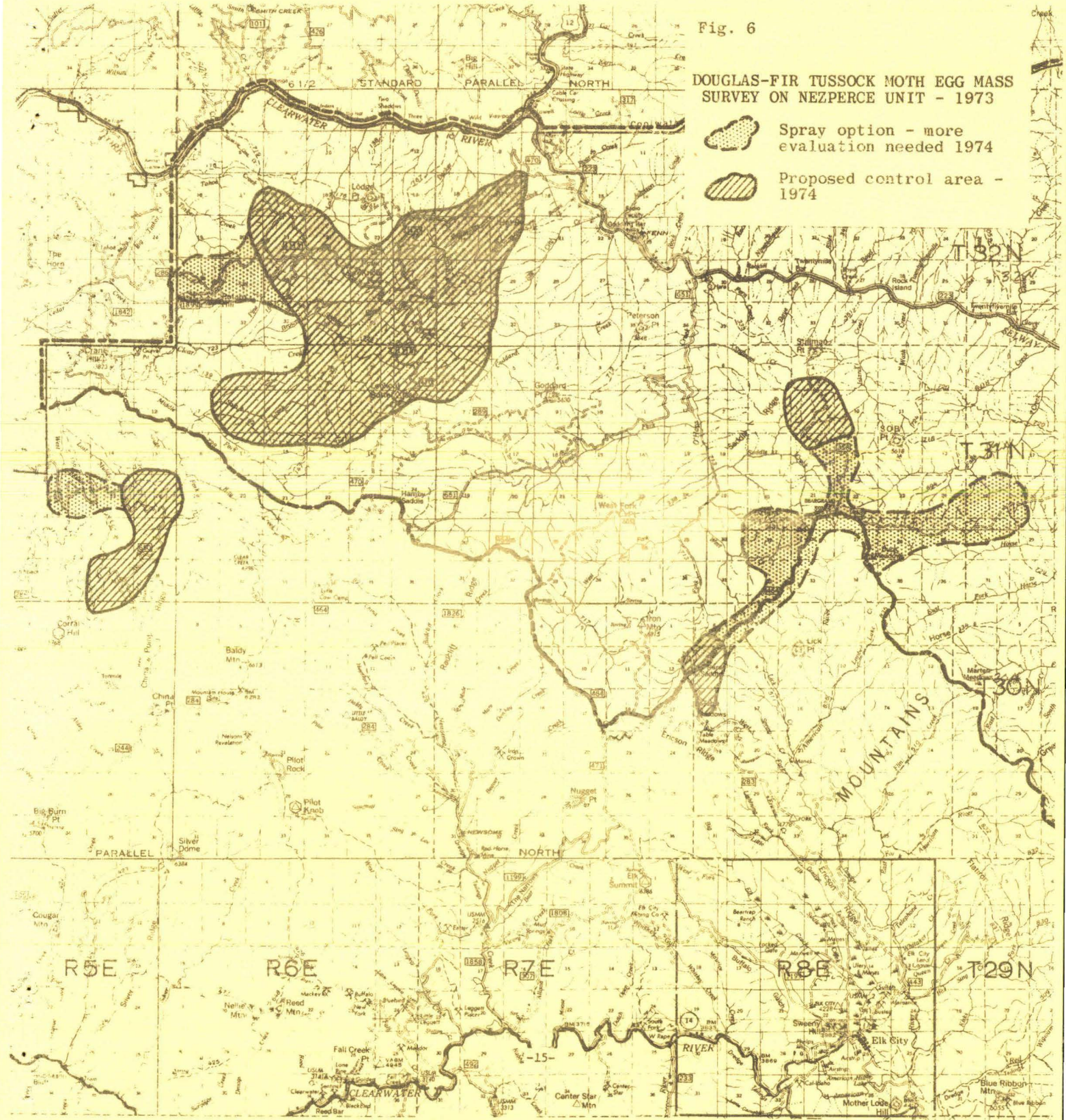


Fig. 6
DOUGLAS-FIR TUSSOCK MOTH EGG MASS SURVEY ON NEZPERCE UNIT - 1973
Spray option - more evaluation needed 1974
Proposed control area - 1974
T32N
T31N
T30N
T29N
R5E
R6E
R7E
R8E
MOUNTAINS
CLEARWATER RIVER
STANDARD PARALLEL NORTH
PARALLEL NORTH
Elk City
Pilot Rock
Silver Dome
Center Star Mtn
Mother Lode Hill
Blue Ribbon Mtn
Fall Creek Pt
Reed Bar
Baldy Mtn
Corral Hill
Nugget Pt
Elk Summit
Sweeny Hill
Reed Mtn
Cougar Mtn
Big Burn Pt
China Pt
Pilot Knob
Ericson Ridge
Peterson Ridge
Goddard Pt
Stillman Pt
Rock Island
Twentymile
Martin Meadows
Table Meadows
Harpster Saddle
Lick Pt
Nelson's Revelator
Mountain House
Iron Crown
Red Horse Ridge
Beartrap Ranch
Buffalo
Blueberry
Loggers Pocket
Lone Pine
Blackbird
Meadow
Rabbit
Orofino
Horse
Ridge
Creek
Fork
-15-

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Appendix Table 1.--Analysis from plots in northern Idaho containing new egg masses in 1973

Unit	Plot location	Egg masses/1,000 sq. in. foliage		Ratio
		New	Old	Old:New
Coeur d'Alene	T. 49 N., R. 3 W., sec. 33	.04 ± .04	0	0:1
	T. 49 N., R. 2 W., sec. 26	.14 ± .14	0	0:2
	T. 49 N., R. 2 W., sec. 32	.01 ± .01	.02 ± .02	1:1
	T. 50 N., R. 2 W., sec. 36	.01 ± .01	0	0:1
	T. 48 N., R. 2 W., sec. 7	.02 ± .02	0	0:1
	T. 49 N., R. 3 W., sec. 36	.03 ± .03	0	0:1
	T. 48 N., R. 3 W., sec. 2	.12 ± .08	0	0:6
	T. 48 N., R. 2 W., sec. 4	.03 ± .03	0	0:1
	T. 49 N., R. 2 W., sec. 2	.01 ± .01	.02 ± .02	1:1
	T. 49 N., R. 2 W., sec. 23	.03 ± .03	0	0:1
	T. 49 N., R. 2 W., sec. 9	.05 ± .03	.02 ± .02	1:2
	T. 49 N., R. 2 W., sec. 8	.09 ± .05	0	0:3
	T. 49 N., R. 2 W., sec. 7	.06 ± .04	.01 ± .01	1:2
	T. 49 N., R. 2 W., sec. 14	.06 ± .03	0	0:4
	T. 49 N., R. 2 W., sec. 12	.02 ± .02	0	0:1
	T. 49 N., R. 2 W., sec. 16	.23 ± .06	.06 ± .03	1:4
	T. 49 N., R. 2 W., sec. 16	.47 ± .08	.12 ± .06	1:4
	T. 49 N., R. 3 W., sec. 25	.73 ± .18	.08 ± .05	1:9
	T. 49 N., R. 3 W., sec. 14	.49 ± .17	.03 ± .03	1:7
	T. 49 N., R. 3 W., sec. 24	.65 ± .18	.02 ± .02	1:35
	T. 48 N., R. 2 W., sec. 5	.01 ± .01	0	0:1
	T. 49 N., R. 2 W., sec. 21	.15 ± .08	0	0:5
	T. 49 N., R. 2 W., sec. 20	.02 ± .02	0	0:1
	T. 49 N., R. 2 W., sec. 22	.14 ± .06	.04 ± .04	1:5
	T. 49 N., R. 2 W., sec. 29	.32 ± .13	.04 ± .04	1:13
	T. 49 N., R. 2 W., sec. 31	.02 ± .02	0	0:1
	T. 49 N., R. 2 W., sec. 29	.01 ± .01	.10 ± .07	1:.3
	T. 48 N., R. 2 W., sec. 6	.01 ± .01	0	0:1
St. Joe	T. 42 N., R. 3 W., sec. 16	.02 ± .02	.01 ± .01	1:2
	T. 43 N., R. 3 W., sec. 22	.44 ± .17	.16 ± .08	1:4.5
	T. 42 N., R. 4 W., sec. 11	.21 ± .09	0	0:5
	T. 43 N., R. 5 W., sec. 22	.28 ± .12	.11 ± .07	1:1.6
	T. 42 N., R. 4 W., sec. 2	.91 ± .33	0	0:17
	T. 44 N., R. 3 W., sec. 18	.02 ± .02	.37 ± .11	1:6.6
	T. 44 N., R. 2 W., sec. 11	.04 ± .02	0	0:2
	T. 43 N., R. 4 W., sec. 5	.52 ± .17	.09 ± .04	1:3
	T. 45 N., R. 4 W., sec. 10	.01 ± .01	.02 ± .02	1:1
	T. 46 N., R. 4 W., sec. 35	.17 ± .08	0	0:4
	T. 43 N., R. 3 W., sec. 17	.07 ± .07	.07 ± .07	1:1
	T. 43 N., R. 4 W., sec. 18	.79 ± .37	.12 ± .09	1:6
	T. 45 N., R. 4 W., sec. 28	.67 ± .18	.11 ± .04	1:5
	T. 43 N., R. 4 W., sec. 6	.02 ± .01	.01 ± .01	1:2

Unit	Plot location	Egg masses/1,000 sq. in. foliage		Ratio
		New	Old	Old:New
St. Joe	T. 44 N., R. 2 W., sec. 1	.08 ± .08	.03 ± .03	1:1
	T. 42 N., R. 3 W., sec. 32	.01 ± .01	.03 ± .03	1:1
	T. 41 N., R. 2 W., sec. 22	.07 ± .07	0	0:1
	T. 43 N., R. 2 W., sec. 28	.18 ± .08	.04 ± .04	1:5
	T. 43 N., R. 2 W., sec. 20	.02 ± .02	0	0:1
	T. 43 N., R. 2 W., sec. 19	.64 ± .17	.02 ± .02	1:22
	T. 40 N., R. 1 E., sec. 12	.08 ± .04	.06 ± .04	1:1
	T. 40 N., R. 4 W., sec. 6	.02 ± .02	.09 ± .09	1:1
	T. 40 N., R. 4 W., sec. 7	.14 ± .07	.15 ± .08	1:1.3
	T. 44 N., R. 3 W., sec. 26	.01 ± .01	0	0:1
	T. 42 N., R. 4 W., sec. 24	.24 ± .10	.22 ± .17	1:1.6
	T. 42 N., R. 4 W., sec. 13	.10 ± .05	.89 ± .24	1:.2
	T. 43 N., R. 3 W., sec. 35	.04 ± .03	0	0:2
	T. 42 N., R. 3 W., sec. 18	.66 ± .31	.11 ± .08	1:4
	T. 43 N., R. 5 W., sec. 27	.11 ± .08	0	0:2
	T. 44 N., R. 3 W., sec. 32	.15 ± .09	.03 ± .02	1:4
	T. 43 N., R. 4 W., sec. 12	.08 ± .05	0	0:4
	T. 42 N., R. 4 W., sec. 1	.07 ± .05	.05 ± .05	1:2
	T. 42 N., R. 3 W., sec. 19	.28 ± .16	.36 ± .16	1:.7
	T. 43 N., R. 4 W., sec. 8	.35 ± .10	0	0:12
	T. 42 N., R. 4 W., sec. 3	.47 ± .12	.04 ± .03	1:6
	T. 42 N., R. 3 W., sec. 6	.18 ± .09	.01 ± .01	1:7
	T. 42 N., R. 3 W., sec. 8	.02 ± .02	0	0:1
	T. 43 N., R. 4 W., sec. 26	.42 ± .30	.16 ± .08	1:2
	T. 42 N., R. 3 W., sec. 29	3.6 ± .53	1.5 ± .35	1:2.5
	T. 43 N., R. 5 W., sec. 12	.03 ± .02	.17 ± .07	1:.2
	T. 43 N., R. 4 W., sec. 17	.34 ± .10	.06 ± .04	1:5
	T. 43 N., R. 5 W., sec. 24	.35 ± .09	.02 ± .02	1:13
	T. 43 N., R. 4 W., sec. 18	.09 ± .05	0	0:3
	T. 45 N., R. 4 W., sec. 2	.09 ± .09	0	0:1
	T. 43 N., R. 4 W., sec. 18	.11 ± .04	.26 ± .07	1:.7
	T. 42 N., R. 4 W., sec. 18	.03 ± .03	0	0:1
	T. 44 N., R. 3 W., sec. 17	.76 ± .21	.06 ± .03	1:8.6
	T. 43 N., R. 4 W., sec. 32	.06 ± .05	.02 ± .02	1:2
	T. 43 N., R. 4 W., sec. 35	.68 ± .23	.11 ± .06	1:5.6
	T. 43 N., R. 3 W., sec. 26	.22 ± .17	.01 ± .01	1:5
	T. 43 N., R. 3 W., sec. 4	.44 ± .13	0	0:12
	T. 43 N., R. 4 W., sec. 33	.02 ± .02	0	0:1
	T. 43 N., R. 1 E., sec. 2	.03 ± .03	.08 ± .06	1:.5
	T. 45 N., R. 4 N., sec. 33	.04 ± .03	.07 ± .04	1:.7
	T. 43 N., R. 4 W., sec. 18	.64 ± .14	.17 ± .05	1:3
	T. 44 N., R. 4 W., sec. 11	.07 ± .04	0	0:4
	T. 45 N., R. 4 W., sec. 21	.12 ± .06	.07 ± .03	1:1.4
	T. 45 N., R. 4 W., sec. 16	.06 ± .04	0	0:4

Unit	Plot location	Egg masses/1,000 sq. in. foliage		Ratio
		New	Old	Old:New
St. Joe	T. 45 N., R. 3 W., sec. 17	.02 ± .02	.05 ± .04	1:.5
	T. 45 N., R. 3 W., sec. 36	.04 ± .04	0	0:1
	T. 45 N., R. 3 W., sec. 15	.09 ± .05	.03 ± .03	1:3
	T. 44 N., R. 3 W., sec. 8	.03 ± .02	0	0:2
	T. 40 N., R. 5 W., sec. 13	.05 ± .03	0	0:2
	T. 41 N., R. 3 W., sec. 5	.04 ± .04	0	0:1
Clearwater	T. 36 N., R. 6 E., sec. 11	.09 ± .09	1.7 ± .70	1:.5
	T. 37 N., R. 3 E., sec. 9	.09 ± .06	.13 ± .07	1:.7
	T. 37 N., R. 4 E., sec. 5	.06 ± .06	0	0:1
	T. 37 N., R. 4 E., sec. 9	.10 ± .07	.04 ± .04	1:2
	T. 37 N., R. 6 E., sec. 23	.05 ± .03	2.5 ± .72	1:.07
Craigmont	T. 33 N., R. 3 W., sec. 29	.31 ± .13	.29 ± .10	1:1.2
	T. 33 N., R. 3 W., sec. 20	.08 ± .06	.08 ± .03	1:.5
	T. 33 N., R. 3 W., sec. 17	.31 ± .13	.29 ± .10	1:1.2
	T. 32 N., R. 4 W., sec. 1	.02 ± .02	.04 ± .04	1:1
Nezperce	T. 23 N., R. 1 W., sec. 29	.51 ± .19	.34 ± .16	1:1.7
	T. 23 N., R. 1 E., sec. 30	.02 ± .02	.29 ± .10	1:.1
	T. 26 N., R. 1 E., sec. 31	.46 ± .35	.05 ± .05	1:4
	T. 25 N., R. 1 E., sec. 31	.54 ± .52	1.2 ± .38	1:.2
	T. 27 N., R. 1 E., sec. 33	1.8 ± 1.2	0	0:2
	T. 31 N., R. 5 E., sec. 24	.10 ± .08	.01 ± .01	1:2
	T. 32 N., R. 6 E., sec. 21	.52 ± .24	.04 ± .04	1:7
	T. 32 N., R. 6 E., sec. 34	.22 ± .11	.08 ± .05	1:2.5
	T. 31 N., R. 8 E., sec. 9	.05 ± .05	0	0:1
	T. 31 N., R. 5 E., sec. 35	.09 ± .04	0	0:4
	T. 30 N., R. 8 E., sec. 7	.06 ± .06	0	0:1
	T. 24 N., R. 1 E., sec. 7	.02 ± .02	0	0:1
	T. 32 N., R. 7 E., sec. 31	.28 ± .16	0	0:3
	T. 24 N., R. 1 W., sec. 6	1.2 ± .26	.36 ± .15	1:2.3
	T. 27 N., R. 2 E., sec. 34	.14 ± .05	0	0:6
	T. 27 N., R. 1 E., sec. 20	.02 ± .02	0	0:1
	T. 26 N., R. 1 E., sec. 19	.56 ± .32	0	0:3
	T. 31 N., R. 7 E., sec. 6	.41 ± .29	.74 ± .34	1:.4
	T. 32 N., R. 6 E., sec. 36	.22 ± .18	0	0:2
	T. 31 N., R. 6 E., sec. 10	.18 ± .09	0	0:4
	T. 32 N., R. 7 E., sec. 17	.10 ± .06	0	0:3
	T. 32 N., R. 6 E., sec. 25	.10 ± .05	0	0:3

Unit	Plot location	Egg masses/1,000 sq. in. foliage		Ratio Old:New
		New	Old	
Nezperce	T. 31 N., R. 6 E., sec. 8	.03 ± .03	0	0:1
	T. 26 N., R. 1 W., sec. 33	1.9 ± .65	.64 ± .35	1:6
	T. 26 N., R. 1 W., sec. 4	2.0 ± .41	.82 ± .19	1:2.4
	T. 26 N., R. 1 E., sec. 7	2.5 ± 1.2	0	0:6
	T. 22 N., R. 1 W., sec. 2	.03 ± .03	1.3 ± .25	1:.03
	T. 25 N., R. 2 W., sec. 13	.40 ± .40	.38 ± .26	1:.5
	T. 26 N., R. 1 W., sec. 3	5.3 ± 3.7	.49 ± .15	1:5.4
	T. 26 N., R. 1 E., sec. 6	.33 ± .21	.02 ± .02	1:3
	T. 23 N., R. 1 W., sec. 27	.48 ± .20	.06 ± .03	1:4.3

Appendix Table 2.--Survey data from plots in northern Idaho containing no new egg masses in 1973

Unit	Plot location	Cocoons only	Old egg masses	No cocoons or egg masses
Coeur d'Alene	T. 51 N., R. 3 W., sec. 30			X
	T. 51 N., R. 3 W., sec. 29	X		
	T. 51 N., R. 3 W., sec. 28	X		
	T. 51 N., R. 3 W., sec. 27	X		
	T. 51 N., R. 3 W., sec. 26	X		
	T. 51 N., R. 3 W., sec. 25			X
	T. 51 N., R. 3 W., sec. 32	X		
	T. 51 N., R. 3 W., sec. 35			X
	T. 50 N., R. 1 W., sec. 18		X	
	T. 50 N., R. 1 W., sec. 20	X		
	T. 50 N., R. 1 W., sec. 21	X		
	T. 50 N., R. 1 W., sec. 30	X		
	T. 50 N., R. 1 W., sec. 28	X		
	T. 50 N., R. 1 W., sec. 31	X		
	T. 50 N., R. 1 W., sec. 33	X		
	T. 50 N., R. 2 W., sec. 34			X
	T. 49 N., R. 2 W., sec. 4	X		
	T. 49 N., R. 2 W., sec. 1		X	
	T. 49 N., R. 1 W., sec. 6	X		
	T. 49 N., R. 1 W., sec. 4	X		
	T. 49 N., R. 1 W., sec. 7	X		
	T. 49 N., R. 1 W., sec. 17		X	
	T. 49 N., R. 2 W., sec. 11	X		
	T. 49 N., R. 2 W., sec. 10	X		
	T. 49 N., R. 3 W., sec. 16			X
	T. 49 N., R. 3 W., sec. 15	X		
	T. 49 N., R. 2 W., sec. 18			X
	T. 49 N., R. 2 W., sec. 19	X		
	T. 49 N., R. 3 W., sec. 21			X
	T. 49 N., R. 3 W., sec. 28	X		
	T. 49 N., R. 3 W., sec. 27			X
	T. 49 N., R. 2 W., sec. 30			X
	T. 49 N., R. 2 W., sec. 28		X	
	T. 49 N., R. 2 W., sec. 27	X		
	T. 49 N., R. 1 W., sec. 31			X
	T. 49 N., R. 2 W., sec. 36			X
	T. 49 N., R. 2 W., sec. 35			X
	T. 49 N., R. 2 W., sec. 34			X
	T. 48 N., R. 2 W., sec. 3	X		
	T. 48 N., R. 3 W., sec. 1	X		
	T. 48 N., R. 3 W., sec. 11			X
	T. 48 N., R. 3 W., sec. 12			X
	T. 48 N., R. 2 W., sec. 8		X	
	T. 48 N., R. 2 W., sec. 17		X	
	T. 48 N., R. 2 W., sec. 18	X		

Unit	Plot location	Cocoons only	Old egg masses	No cocoons or egg masses
St. Joe	T. 44 N., R. 1 E., sec. 23	X		
	T. 44 N., R. 2 E., sec. 30	X		
	T. 44 N., R. 2 E., sec. 32			X
	T. 43 N., R. 2 E., sec. 12			X
	T. 43 N., R. 2 E., sec. 24		X	
	T. 43 N., R. 5 W., sec. 26			X
	T. 43 N., R. 4 W., sec. 28			X
	T. 43 N., R. 4 W., sec. 25	X		
	T. 43 N., R. 3 W., sec. 34	X		
	T. 42 N., R. 2 W., sec. 9	X		
	T. 42 N., R. 3 W., sec. 5	X		
	T. 42 N., R. 3 W., sec. 10	X		
	T. 42 N., R. 3 W., sec. 9	X		
	T. 42 N., R. 3 W., sec. 7	X		
	T. 42 N., R. 4 W., sec. 8			X
	T. 42 N., R. 4 W., sec. 9	X		
	T. 42 N., R. 4 W., sec. 16	X		
	T. 42 N., R. 4 W., sec. 14	X		
	T. 42 N., R. 3 W., sec. 17			X
	T. 42 N., R. 3 W., sec. 22	X		
	T. 42 N., R. 3 W., sec. 21	X		
	T. 42 N., R. 3 W., sec. 20	X		
	T. 42 N., R. 4 W., sec. 23			X
	T. 42 N., R. 4 W., sec. 21	X		
	T. 42 N., R. 3 W., sec. 27	X		
	T. 42 N., R. 3 W., sec. 28	X		
	T. 42 N., R. 3 W., sec. 30	X		
	T. 42 N., R. 4 W., sec. 27			X
	T. 42 N., R. 3 W., sec. 33	X		
	T. 42 N., R. 3 W., sec. 34	X		
	T. 42 N., R. 3 W., sec. 35	X		
	T. 41 N., R. 3 W., sec. 4			X
	T. 41 N., R. 2 W., sec. 7			X
	T. 41 N., R. 2 W., sec. 9			X
	T. 41 N., R. 2 W., sec. 17	X		
	T. 41 N., R. 2 W., sec. 28	X		
	T. 41 N., R. 3 W., sec. 20			X
	T. 41 N., R. 5 W., sec. 23	X		
	T. 41 N., R. 5 W., sec. 21			X
	T1 41 N., R. 5 W., sec. 29			X
	T. 40 N., R. 5 W., sec. 10			X
	T. 40 N., R. 5 W., sec. 11	X		
	T. 40 N., R. 4 W., sec. 5	X		
	T. 40 N., R. 4 W., sec. 4	X		
	T. 40 N., R. 4 W., sec. 3	X		
	T. 40 N., R. 4 W., sec. 1	X		

Unit	Plot location	Cocoons only	Old egg masses	No cocoons or egg masses
St. Joe	T. 46 N., R. 4 W., sec. 29		X	
	T. 46 N., R. 4 W., sec. 26	X		
	T. 45 N., R. 5 W., sec. 10		X	
	T. 45 N., R. 3 W., sec. 12	X		
	T. 45 N., R. 4 W., sec. 20	X		
	T. 45 N., R. 4 W., sec. 32	X		
	T. 45 N., R. 3 W., sec. 31		X	
	T. 44 N., R. 2 W., sec. 3	X		
	T. 44 N., R. 2 W., sec. 6	X		
	T. 44 N., R. 3 W., sec. 4	X		
	T. 44 N., R. 3 W., sec. 6	X		
	T. 44 N., R. 4 W., sec. 3		X	
	T. 44 N., R. 4 W., sec. 4			X
	T. 44 N., R. 3 W., sec. 15	X		
	T. 44 N., R. 3 W., sec. 14			X
	T. 44 N., R. 2 W., sec. 18	X		
	T. 44 N., R. 2 W., sec. 14	X		
	T. 44 N., R. 2 W., sec. 20	X		
	T. 44 N., R. 3 W., sec. 22	X		
	T. 44 N., R. 5 W., sec. 28			X
	T. 44 N., R. 3 W., sec. 33	X		
	T. 44 N., R. 3 W., sec. 35			X
	T. 43 N., R. 1 W., sec. 16		X	
	T. 43 N., R. 2 W., sec. 9	X		
	T. 43 N., R. 3 W., sec. 5			X
	T. 43 N., R. 3 W., sec. 9	X		
	T. 43 N., R. 4 W., sec. 13	X		
	T. 43 N., R. 4 W., sec. 24		X	
	T. 43 N., R. 3 W., sec. 29		X	
	T. 43 N., R. 5 W., sec. 2		X	
	T. 43 N., R. 4 W., sec. 4	X		
	T. 43 N., R. 4 W., sec. 7	X		
	T. 43 N., R. 5 W., sec. 9			X
	T. 43 N., R. 5 W., sec. 13		X	
	T. 43 N., R. 5 W., sec. 14		X	
	T. 43 N., R. 4 W., sec. 19	X		
	T. 43 N., R. 4 W., sec. 21	X		
	T. 45 N., R. 1 W., sec. 24			X
	T. 45 N., R. 1 E., sec. 27		X	
	T. 45 N., R. 1 E., sec. 33	X		
	T. 45 N., R. 1 E., sec. 34		X	
	T. 44 N., R. 1 E., sec. 4	X		
	T. 44 N., R. 1 E., sec. 1	X		
	T. 44 N., R. 1 E., sec. 10		X	
	T. 44 N., R. 1 E., sec. 11	X		

Unit	Plot location	Cocoons only	Old egg masses	No cocoons or egg masses
St. Joe	T. 40 N., R. 4 W., sec. 10	X		
	T. 40 N., R. 4 W., sec. 11	X		
	T. 40 N., R. 4 W., sec. 12	X		
	T. 40 N., R. 4 W., sec. 20			X
	T. 40 N., R. 4 W., sec. 22	X		
	T. 40 N., R. 4 W., sec. 24			X
	T. 40 N., R. 4 W., sec. 26	X		
	T. 40 N., R. 3 W., sec. 6		X	
	T. 40 N., R. 3 W., sec. 5	X		
	T. 40 N., R. 3 W., sec. 4	X		
	T. 40 N., R. 3 W., sec. 8	X		
	T. 40 N., R. 3 W., sec. 11	X		
	T. 40 N., R. 3 W., sec. 17	X		
	T. 40 N., R. 3 W., sec. 21	X		
	T. 43 N., R. 2 W., sec. 35		X	
	T. 43 N., R. 1 W., sec. 31		X	
	T. 42 N., R. 1 W., sec. 5		X	
	T. 42 N., R. 1 W., sec. 1		X	
	T. 42 N., R. 1 W., sec. 15		X	
	T. 42 N., R. 1 W., sec. 32	X		
	T. 42 N., R. 1 E., sec. 11	X		
	T. 42 N., R. 1 E., sec. 29	X		
	T. 42 N., R. 3 E., sec. 9			X
	T. 42 N., R. 3 E., sec. 17			X
	T. 42 N., R. 3 E., sec. 29		X	
	T. 41 N., R. 3 E., sec. 30			X
	T. 41 N., R. 2 E., sec. 11	X		
	T. 41 N., R. 2 E., sec. 10	X		
	T. 41 N., R. 2 E., sec. 7	X		
	T. 41 N., R. 2 W., sec. 11	X		
	T. 41 N., R. 2 W., sec. 13	X		
	T. 40 N., R. 2 W., sec. 14	X		
	T. 40 N., R. 1 W., sec. 7	X		
	T. 40 N., R. 1 E., sec. 20			X
	T. 40 N., R. 1 E., sec. 28			X
	T. 40 N., R. 2 E., sec. 4			X
	T. 40 N., R. 3 E., sec. 6			X
	T. 40 N., R. 3 E., sec. 17	X		
	T. 40 N., R. 3 E., sec. 20			X
	T. 40 N., R. 3 E., sec. 29			X
	T. 40 N., R. 3 E., sec. 35			X
	T. 39 N., R. 3 E., sec. 17			X
	T. 39 N., R. 3 E., sec. 19			X
	T. 39 N., R. 2 E., sec. 7			X
	T. 39 N., R. 2 E., sec. 17			X
	T. 39 N., R. 2 E., sec. 16			X

Unit	Plot location	Cocoons only	Old egg masses	No cocoons or egg masses
St. Joe	T. 39 N., R. 2 E., sec. 20			X
	T. 39 N., R. 1 E., sec. 35			X
	T. 39 N., R. 1 W., sec. 22			X
	T. 39 N., R. 1 W., sec. 27			X
	T. 38 N., R. 1 W., sec. 5			X
	T. 38 N., R. 1 E., sec. 4			X
	T. 38 N., R. 1 E., sec. 8			X
	T. 38 N., R. 1 E., sec. 17			X
	T. 38 N., R. 1 E., sec. 19			X
	T. 38 N., R. 1 E., sec. 29			X
Clearwater	T. 39 N., R. 4 E., sec. 27	X		
	T. 38 N., R. 4 E., sec. 28			X
	T. 38 N., R. 4 E., sec. 32	X		
	T. 37 N., R. 4 E., sec. 1	X		
	T. 37 N., R. 4 E., sec. 10	X		
	T. 37 N., R. 4 E., sec. 13	X		
	T. 37 N., R. 3 E., sec. 2	X		
	T. 37 N., R. 3 E., sec. 5			X
	T. 37 N., R. 3 E., sec. 7			X
	T. 37 N., R. 3 E., sec. 16			X
	T. 37 N., R. 3 E., sec. 30	X		
	T. 37 N., R. 1 E., sec. 24			X
	T. 36 N., R. 4 E., sec. 21			X
	T. 36 N., R. 4 E., sec. 23			X
	T. 39 N., R. 5 E., sec. 15	X		
	T. 39 N., R. 5 E., sec. 21	X		
	T. 39 N., R. 5 E., sec. 33		X	
	T. 38 N., R. 6 E., sec. 13		X	
	T. 38 N., R. 7 E., sec. 7		X	
	T. 38 N., R. 7 E., sec. 22		X	
	T. 37 N., R. 7 E., sec. 5		X	
	T. 37 N., R. 7 E., sec. 22		X	
	T. 37 N., R. 7 E., sec. 33		X	
	T. 37 N., R. 6 E., sec. 32		X	
	T. 36 N., R. 7 E., sec. 17	X		
	T. 36 N., R. 7 E., sec. 31		X	
	T. 35 N., R. 7 E., sec. 9	X		
Craigmont	T. 33 N., R. 3 W., sec. 9	X		
	T. 33 N., R. 3 W., sec. 16	X		
	T. 33 N., R. 3 W., sec. 32	X		
	T. 33 N., R. 3 W., sec. 33		X	

Unit	Plot location	Cocoons only	Old egg masses	No cocoons or egg masses
Craigmont	T. 33 N., R. 4 W., sec. 35	X		
	T. 33 N., R. 2 W., sec. 17			X
	T. 32 N., R. 4 W., sec. 2		X	
	T. 32 N., R. 4 W., sec. 36		X	
	T. 32 N., R. 3 W., sec. 5	X		
	T. 32 N., R. 3 W., sec. 9	X		
	T. 32 N., R. 3 W., sec. 15	X		
	T. 32 N., R. 3 W., sec. 21	X		
	T. 32 N., R. 3 W., sec. 22	X		
	T. 32 N., R. 3 W., sec. 26			X
	T. 32 N., R. 3 W., sec. 27	X		
	T. 32 N., R. 3 W., sec. 28	X		
Nezperce	T. 32 N., R. 6 E., sec. 16		X	
	T. 32 N., R. 6 E., sec. 24	X		
	T. 32 N., R. 6 E., sec. 30	X		
	T. 32 N., R. 6 E., sec. 29	X		
	T. 32 N., R. 6 E., sec. 31			X
	T. 31 N., R. 5 E., sec. 3	X		
	T. 31 N., R. 5 E., sec. 22	X		
	T. 31 N., R. 6 E., sec. 12	X		
	T. 31 N., R. 6 E., sec. 24			X
	T. 31 N., R. 6 E., sec. 34	X		
	T. 31 N., R. 7 E., sec. 19	X		
	T. 31 N., R. 7 E., sec. 23	X		
	T. 31 N., R. 7 E., sec. 30			X
	T. 31 N., R. 7 E., sec. 33			X
	T. 31 N., R. 8 E., sec. 15	X		
	T. 31 N., R. 8 E., sec. 29		X	
	T. 31 N., R. 8 E., sec. 28	X		
	T. 31 N., R. 9 E., sec. 19	X		
	T. 30 N., R. 7 E., sec. 11			X
	T. 30 N., R. 6 E., sec. 4			X
	T. 30 N., R. 6 E., sec. 9			X
	T. 28 N., R. 1 W., sec. 14			X
	T. 28 N., R. 1 W., sec. 13	X		
	T. 28 N., R. 1 W., sec. 25			X
	T. 27 N., R. 2 E., sec. 35		X	
	T. 25 N., R. 1 W., sec. 6		X	
	T. 25 N., R. 2 E., sec. 28			X
	T. 25 N., R. 2 E., sec. 25			X
	T. 25 N., R. 2 E., sec. 36			X
	T. 24 N., R. 1 W., sec. 26	X		
	T. 24 N., R. 1 W., sec. 27		X	
	T. 24 N., R. 1 W., sec. 33			X
	T. 23 N., R. 1 W., sec. 20	X		

APPENDIX - SECTION D

Background Document on *Bacillus thuringiensis*

I. General Information

A. Common Name

This pesticide is commonly referred to as Bt. The name derives from the initials of the active ingredient *Bacillus thuringiensis* , an aerobic spore-forming, crystal-producing member of the bacterial genus *Bacillus*.

B. Chemical Name

There is no chemical name for this pesticide since it is based on a living bacterium.

C. Registered Uses

In terms of forest insect pest control, Bt products are registered for use against the following insects (ground application only)

a. Spring and fall cankerworm.
b. Great Basin tent caterpillar.
c. Fall webworm.
d. California oakmoth.
e. Red-humped caterpillar.
f. Gypsy moth.
g. Elm spanworm.

D. Formulations Manufactured

Bt is available in three basic formulations:

a. Wettable powder
b. Aqueous concentrate
c. Dust

E. Dilution of Formulations for Use.

Water is the generally accepted diluent for wettable powder and aqueous concentrate formulations. Glycol and oils have been used experimentally, but were generally not as good as water for a diluent. Dust formulations are not diluted.

F. Rates and Methods of Application.

At present, the only registered method of application is by ground equipment for forest pests (hydraulic and mist blower).

Rates of application of the active ingredient, depending upon the insect species, range from 0.25 to 4 pounds of wettable powder and 0.50 to 2 quarts of aqueous concentrate per acre.

G. Tolerances in Food.

All Bt products are exempt from tolerance limits. (See Federal Register Vol. 36(228) section 180.1011.)

H. Manufacturer

a. Bactospeine* - French manufactured and registered. Not registered in U.S. Distributed in U.S. by Rhodia, Inc. Chipman Div.

b. Biotrol XK - EPA Temporary permit No. 6296-EXP-4G.

Manufactured by Nutrilite Products, Inc., Lakeville, California 92353. Distributed by Thompson-Hayward Chemical Co., Kansas City, Kansas 66110.

c. Dipel - EPA Registration No. 275-18.

Manufactured and distributed by Agircultural and Veterinary Products Division, Abbott Laboratories, North Chicago, Illinois 60064.

d. Thuricide-HPC - EPA Registration No. 4456-541.

Manufactured and distributed (bulk) by International Minerals and Chemical Corp., Libertyville, Illinois.

II. Toxicity data on formulations

A. Safety data

1. Acute Mammalian Studies

a. Thuricide was administered to rats in a 33 percent suspension in water with carboxymethyl cellulose as a thickener. The dose was placed in the stomach by means of a ball-tipped hypodermic syringe. Doses up to 24 grams of Thuricide (2×10^{12} spores/kilogram) were administered to groups of 10 rats. The animals were observed for 1 week. No fatalities occurred. No outward symptoms of toxicity. Gross and histological examination of tissues showed no differences from tissues of control animals. (1,2)

*Trademark of Pasteur Institute, used by Laboratory Roger Bellon.

Eighteen humans ingested 1 gram of Thuricide daily for 5 days. Complete physical and laboratory examinations were given prior to the experiment, at the end of the 5-day ingestion period, and 4 to 5 weeks later. Physical examinations included detailed history and records of height, weight, temperature, blood pressure, respiratory rate, pulse rate immediately after exercise and 30 and 60 seconds thereafter. Evaluations were made of genitourinary, gastrointestinal, cardiorespiratory, and nervous systems. Laboratory tests included routine urinalysis with qualitative and quantitative urobilinogen determinations (when indicated), complete blood count, sedimentation rate, blood urea N, glucose, bilirubin, and thymol turbidity tests. All subjects remained well during the course of the experiment. All laboratory findings were negative. (1)

Dipel was fed to groups of 10 mice (16 to 25 grams) at the rate of 10 grams/kilogram (1.5×10^7 I.U.). No mortality occurred. LD_{50} was beyond 10 grams/Kg. (11)

Dipel was fed to three female mongrel dogs. Dosage was 400 mg/kg (6×10^6 I.U./Kg). The animals were sympton free during the 48-hour observation period. (11)

b. Dermal

Dermal effects of Bt were tested by application to shaved flanks and bellies of albino rabbits. Dosages used ranged from 20 percent suspensions to 50 mg/animal. After application half of the treated skin was abraded while the other half was left intact. Readings were made at 24, 48, and 72 hours in one test and up to 3 weeks in another. Other than local, mild erythema, no ill effects were noted in any test animal. (1,2)

In another study dermal application to albino rabbits was made to test allergenicity response. Ten sensitizing doses were applied every other day for 3 weeks. Readings were made 24 hours after each application of Bt. Two weeks after the 10th application a challenge dose was applied. Only slight erythema and edema were noted. No allergenic response was elicited. (1)

Allergenicity was also tested with guinea pigs following the procedure of Draize. No allergenic response was noted. (1)

c. Inhalation

Bt inhalation studies were conducted on mice, rats, guinea pigs, and human volunteers.

In one test with mice, the animals were exposed to 10 grams of Bt powder for 15 minutes. Dosages were applied four times over a period of 6 days. No ill effects were noted and gross pathology was negative. (1)

In other tests with rats and guinea pigs, exposure to a 10 percent Bt preparation for 10 minutes, no fatalities were recorded for the 1 week observation time. Dyspnea (discomfort) was noted but recovery was rapid. The animals showed normal weight grain. (1)

Five human volunteers inhaled 100 mg Bt powder daily for 5 days. Complete physical examinations prior to the test, immediately after the test, and 4 to 5 weeks later showed no abnormal conditions in the test subjects. (1)

d. Eye irritation

Ocular irritation with Bt was tested in albino rabbits. 0.1 cc of a 20 percent suspension was instilled in each eye. One eye was immediately rinsed with isotonic saline. Six animals were tested. The eyes were examined immediately, after 3 hours and 24 hours, and every 24 hours until they appeared normal. Slight redness of the eyelids was noted at 3 hours and 24 hours. Eye irritation disappeared in 48 hours. (1)

2. Subacute and chronic studies

Bt daily administered as the spore-crystal complex to rats for 3 months at per os rates of 25, 100, and 400 mg/kg produced no main function disorders or organ damage. Similar results were obtained in dogs fed 6, 25, and 100 mg/kg for 3 months. (1,2)

3. Other Studies

a. Birds

Avian and fish toxicity tests have been conducted with several Bt preparations.

One long term study with six New Hampshire Red laying hens was conducted over a 23-month period. The hens received a daily dose of Bt ranging from 0.5 to 10 grams per bird. Results showed no allergic response, other illnesses, or

variations in the expected egg production of the hens on test diet. There was no significant difference between the birds and the birds used as controls.

In a 9-week oral toxicity test administered to 24 groups of 10 chicks each, no significant differences of any kind were noted between the test and control groups of chicks. (12)

Seventeen pheasants and two partridges, all about 6 weeks old, were divided into two groups. One group was fed 1.0 gram of Bt/bird/day in two gelatin capsules. The control groups were fed two empty gelatin capsules daily. (4)

No deaths or symptoms of respiratory, alimentary or other disturbances were noted in the Bt fed group. Two pheasants in the control group died of trauma (due to handling). Birds in both groups exhibited feather color and pattern, bearing, and weight gain that are expected in similar groups of birds in nature.

It was concluded that no differences in behavior or development of test birds resulted from the ingestion of 1.0 gram of Bt spore-crystal complex per bird per day when compared to the control group.

In an oral acute toxicity study conducted with Bt in young adult bobwhite quail showed the acute oral medial lethal dose to be in excess of 10 gm/kg body weight. Five male and five female quail were fed 10 gm/kg by gavage. A similar group was fed distilled water as control (12,13)

The test period was 21 days. At the end of the test period all animals were sacrified and subjected to a gross pathological examination. No pathology attributable to the test material was found. Growth rate was similar in the test and control groups.

b. Fish

Several acute fish toxicity tests were conducted with Bt preparations.

A 4-day fish toxicity study was conducted with Bt employing rainbow trout and bluegills as test animals. Two groups of 10 fish each were placed in water containing Bt at concentrations of 560 and 1,000 p.p.m. As a check each lot of experimental fish was challenged with a reference pesticide, pp^1-DDT. None of the trout or bluegills exposed 96 hours to Bt at concentrations of 560 and 1,000 p.p.m. died. The 96 median tolerance limits (TL_{50}) of pp^1-DDT for the same lots of fish were calculated to be 0.024 p.p.m. for trout and 0.032 p.p.m. for bluegills. (1)

In another test with rainbow trout, results showed no acute effects on 2.5-inch trout exposed to 1 to 10 mg Bt/kg water. (1)

Larger rainbow trout, 4 inches in length, were exposed to Bt at concentrations of 100 to 1,000 p.p.m. for 14 days. No deaths or symptoms of alimentary or behavioral disturbances were evident. (1)

In a test with juvenile Coho salmon (1.6 inches in length), Bt was about 1/30 as toxic as DDT. The tests ran for 168 hours with concentrations of 8 to 406 mg Bt/1. water. (1)

The 48-hour median tolerance limit of the Bt was about 50 mg/1. whereas that for DDT was 0.08 mg/1 water.

c. Effects on beneficial insects

(1) Bees

Numerous tests with several serotypes of Bt showed no deletrious effects where practical dosages were used. Heat stable exotoxin is poisonous to bees as is high concentrations of spores. (15)

(2) Silkworm

This domesticated insect is highly susceptible to Bt particularly var. *thuringiensis*. Because of this, it is illegal to disseminate foreign preparations of any pathogen in Japan. (15)

B. Physical - Chemical Properties

Inasmuch as all commercially available Bt products are biological materials, they do not have all the physico-chemical properties of chemical insecticides.

Bactospeine, Biotrol, and Dipel are marketed as wettable powders or dusts and do not have boiling points, flash points, or vapor pressure. All three materials are stable for more than a year at commercial warehouse or laboratory conditions. These wettable powders are insoluble in normally used carriers such as water, spray oils, etc. However the primary active ingredient, the crystal, will solubilize under high pH conditions (9.5 plus).

Thuricide HPC, the fourth commercial product available in the U.S., is marketed as an aqueous suspension. It must be protected from freezing and high temperatures (above 90°F). The product is stable for several months. Turicide has no boiling point or flash point. Its density is 1.07 at 90°C. The material steams off without boiling and the solid material remaining decomposes without melting.

III. Efficacy data under field and laboratory conditions

Most of the laboratory and field efficacy data were obtained on preparations of Bt based serotype I strains. U.S. produced Bt preparations are no longer based on serotype I strains. A brief resume of field efficacy of these earlier tests against forest insects is given below.

Gypsy moth

Reported field trails in the period 1961-66, indicated improved results in attempts to control the gypsy moth with Bt. These improved results were primarily due to improvements in the commercially available formulations. Evaluations were based primarily on population reduction (egg mass reduction or direct larval mortality) or on foliage protection. The consensus of these reports indicated variable results, particularly from aerial treatment. Some foliage protection was noted, but populations of the insect were not reduced to the levels desired. (8,9)

Spruce budworm

In 1961-65 field tests with Bt in Canada and the United States indicated that unacceptable population control could be achieved by Bt. Evaluations were based primarily on population reduction (larval and pupal mortality and residual egg masses). (16)

Linden looper

A 1962 field test using Bt products resulted in 100 percent control on the linden looper.

Great Basin tent caterpillar

A large-scale field test with Bt against this tent caterpillar gave acceptable population reduction results. Populations were 95 percent lower in treated areas as compared to untreated areas 1 year after application.

California oak moth

Field tests with Bt against this insect gave acceptable population reductions results. Complete control of the insect was reported.

Laboratory tests

Virtually all major forest insect lepidopterous pests proved to be susceptible to the killing effect of Bt. However, many of the field tests resulting from favorable laboratory tests failed or gave unacceptable control effects. The reasons for these field failures are usually ascribed to:

1. Lack of persistence in the field.

2. Poor application techniques.

3. Effects of environmental factors such as U.V., rain, foliage effects.

4. Poor formulations.

5. Instability of product.

6. Inadequate coverage particularly from the air.

7. Insufficient field effects of the Bt strain used.

In 1970, Bt for the control of insects took a new turn. A new isolate HD-1, identified as a variety *Kurstaki* serotype III, was propagated and exhibited a much greater degree of effectiveness than earlier preparations.

Laboratory tests showed that the new isolate was 10 to 100 times more effective than commercial preparations based on the serotype I, Berliner strain.

This new isolate was put into commercial production and now forms the basis for the three commercially available U.S. products.

The first laboratory and field efficacy trials with these new products were conducted against agricultural crop pests, such as the cabbage looper. Crop damage evaluations and population reductions compared favorably with chemical insecticide controls.

Laboratory evaluations of these new products gave encouraging data, so that several field tests were conducted in 1971 against selected forest insect pests, such as the gypsy moth, spruce budworm, elm spanworm, saddled prominent, and hemlock looper. Although the results of these field trials have not appeared in print, a summary of some of the results can be made.

Gypsy Moth

Field efficacy tests were conducted in several areas in the Northeast. Ground mist blower tests using Bt at 8 and 16 billion International Units per acre gave excellent foliage protection in all cases and some degree of population control as measured by egg mass changes (personal communication, Metterhouse, W.; Becker, W.; Yendol, W.; and Dubois, N.). Applications were made three times at 10-day intervals.

An aerial application of the new Bt was reported as highly successful in population reduction and foliage protection (personal communication, Secrest, J.). Two applications of 8 billion International Units per acre in 1 gallon of water were used. A molasses-based adjuvant was used in this new formulation.

Ground application of the new Bt products in water only gave excellent control of the elm spanworm (personal communication, Kaya, H.).

Foliage protection was achieved against the gypsy moth, but population reductions were not acceptable.

Spruce budworm

Aerial application of the new Bt in an experimental formulation gave excellent foliage protection and population reduction when applied twice against the spruce budworm in Canada (personal communication, Smirnoff, W.).

The formulation tested in Canada was glycol based and was applied by spinning disc nozzles.

It should be pointed out that field efficacy tests against forest insect pests with the new Bt strain and improved, or experimental formulations started in 1971. Considerable more field testing will be done in 1972 against more forest pests.

The data from the 1971 tests indicate clearly that the new Bt preparation can readily achieve one control purpose, i.e., foliage protection.

Preliminary data from aerial tests of Dipel and Thuricide in 1972 gave indications of good foliage protection but relatively unsatisfactory population reductions of the gypsy moth. More work must be done with dosage, timing, and rates of application before a full evaluation of Bt can be made.

Phytotoxicity

No phytotoxicity has been reported for the new formulations of Bt.

In some cases, earlier formulations of Bt showed some phytotoxicity, but this was ascribed to adjuvants rather than the active ingredient.

Translocation

There have been no reports of Bt translocation in plants. Since the active ingredient is particulate and insoluble in commonly used carriers, translocation would be impossible. Persistence in soil, water, and plants.

There is a scarcity of hard data on the longevity of Bt in the environment. The studies that have been made indicate that the spore will persist in soil for several weeks depending upon the soil type, soil flora, and extrinsic factors such as pH, moisture, and radiation.

No data is available on the survival of the crystal. However, since the crystal is proteinaceous, enzymatic degradation by soil flora action can be presumed.

Survival of Bt on leaves is minimal when no additives are included in sprays. (17). Biological activity is usually restricted to 3 to 5 days. However, new formulations designed to protect Bt from the ravages of environmental factors have shown considerable biological activity after 21 days of field exposure. Biological activity after 1 month is negligible.

Compatibility with other chemicals

Bt is incompatible with any chemical having a high pH (9.5 plus). The Bt products produced in the United States are compatible with most of the commonly used insecticides and fungicides. However, the manufacturer of Bactospeine does not recommend that the material be mixed with other pesticides.

IV. Environmental Impact

A. In view of the body of safety data regarding the effects of Bt on humans, domestic animals, fish, birds, and beneficial insects which indicated that no ill effects were suffered by test animals, no monitoring of Bt used in forest insect control has been reported.

B. Inasmuch as Bt is exempted from tolerance, no residue analysis on food or feed has been performed when Bt has been used for forest insect control.

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APPENDIX - SECTION E

PRODUCTION, SAFETY EVALUATION, AND INFECTIVITY TITER ASSAY OF A NUCLEOPOLYHEDROSIS VIRUS PREPARATION FOR FIELD APPLICATION

by

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TABLE OF CONTENTS

SUMMARY iii

RECOMMENDATIONS iv

INTRODUCTION 1

PRODUCTION OF THE VIRAL SUSPENSION 1

Substrate 1
Recovery of the polyhedral inclusion bodies 2
Enrichment of the polyhedral inclusion bodies 3
Formulation, packaging, and storage 4
Yield 4

SAFETY EVALUATION 5

Toxicity-pathogenicity study 5
Production control 7

INFECTIVITY TITER 8

PLANNING AND COORDINATION 10

COOPERATORS 10

LITERATURE CITED 10

FIGURES

Figure 1. Flow diagram of the production of the viral suspension 11

TABLES

Table 1. Infectivity titers of three samples of preparation 49 9

SUMMARY

A nucleopolyhedron preparation sufficient for treating 206 acres of fir forest at 10^{11} viral inclusion bodies per acre was produced by a simplified procedure. The method consisted of 3 principal steps: (1) propagation of the virus in Douglas-fir tussock moth larvae; (2) recovery of the inclusion bodies by homogenizing the larval tissues and by passage of the suspension through a series of sieves; and (3) enrichment of the inclusion-body suspension by centrifugation in a sucrose solution.

Tests to determine the safety of the viral agents for bees, fish, and mammals were completed, and gave no indication that the preparation would be unsafe for use as an insecticide. The tests were reviewed by public-health virologists. Routine production control tests confirmed that pathogenic agents (such as *Shigella* and *Salmonella*) were absent from the batch actually used in the field.

Virus production and production control were strictly coordinated as outlined in a flow diagram which shows the sequence and interaction of the various phases of our work.

RECOMMENDATIONS

1. Our present laboratory facilities and instrumentation are not geared for the efficient production of batches of virus in excess of 100-acre doses. Large-scale production facilities and processing equipment, as well as specialized technicians are needed for the production of larger batches of virus for field use. Contracts with the industry or with the federal Pine Bluff biological laboratory should be given serious consideration in the future.
2. Contamination with undesired viruses should be rigorously avoided during the propagation of the intended viral agent in larvae. This could be achieved, e.g., by utilizing an inbred laboratory strain of the host insect rather than field-collected insects.
3. The infectivity titer of the viral preparation should be checked for a two-day period in the field, following application. Infectivity titer losses caused by environmental factors (especially solar radiation) should be determined by assay of samples eluted from plates or foliage at 0^{hr}, 24^{hr}, and 48^{hr} after spraying.

INTRODUCTION

Virus suspensions for field applciations should have the following basic characteristics:

(1) High specific activity titer

(2) Low content of insect tissue components

(3) Low level of bacterial contamination

(4) Absence of toxicity and pathogenicity for mammals, fish, bees, and plants.

During the early part of 1971, we prepared a suspension of viral inclusion bodies (our virus preparation No. 49) to comply with the requirements listed above. A description of the preparation and a summary of the production control tests follows. Our study included the assay of samples of the virus suspension obtained at the spray-system nozzles, in the field. This assay would serve the purpose of estimating the short-term stability of the virus in the complete spray formulation, in a helicopter spray system, under actual field conditions.

PRODUCTION OF THE VIRAL SUSPENSION

Substrate

The virus was produced *in vivo*, in large-scale rearings of larvae of *Hemerocampa pseudotsugata*, during 1966, 1967, and 1968. The total fresh weight of the larvae used for this preparation was kg 10.238, or lbs 22.57. Of this amount, kg 5.492 were lyophilized, whereas kg 4.746

were frozen, but not lyophilized. Both batches were preserved at -18°C up to the time of processing. Although, the number of dead larvae used for this preparation was not recorded, based on the average fresh weight at death (150 mg per larva) we estimate that a total of 113,800 larvae were inoculated with virus and that 68,300 (60%) fo these were utilized for the viral preparation (pupae and larvae which died prematurely were excluded from the batches at harvest time). When ready for inoculation, the larvae were allowed to feed on medium containing 10^6 polyhedral inclusion bodies (PIB) per ml. Occasionally, other procedures were used on some of the batches, e.g., microfeeding by means of glass microcatheters and intrahemocoelic inoculation of virions.

Recovery of the polyhedral inclusion bodies

The dead larvae were separated into several batches and ground at high speed, for 2 min., in a gallon-size Waring blender with a jacketed cooling base. A buffer (0.01 M tris, pH 7.1) and a wetting agent (0.00075% sodium dioctyl sulfosuccinate) were added during the grinding process. The resulting suspension was passed through four stainless steel sieves (48, 100, 200, and 270 meshes to the inch). The final volume of the pooled suspension, after elimination of the larger particles, was 14.8 liters. Twenty-one percent of insoluble solids were eliminated by this screening process.

Enrichment of the polyhedral inclusion bodies

In previous experiments we established that the weight of the inclusion bodies obtained from tussock moth larvae inoculated with nucleopolyhedrosis virus amounts to 6.5% of their total residue (TR), at 104°C. Thus, we could predict a yield of about 85 grams of pure polyhedral inclusion bodies from the raw material available. Therefore, 93.5% of the TR present in the homogenate was to be eliminated by a selective procedure, without excessive loss of PIBs. This was achieved by centrifugation in a sucrose solution of appropriate specific gravity (Martignoni, Iwai, and Breillatt, 1971).

The specific gravity of the screened suspension was adjusted to 1.2363 g/cm^3 with the addition of kg 13.34 of cane sugar (51.2% of the suspension, w/w). The pH was stabilized with additional tris buffer, to a molarity of 0.05. This suspension (22.9 liters) was centrifuged, in 250 ml aliquots, at 13,200 g in a GSA rotor, for 1 hr at 20°C (1,500 ml per run). The sediments were collected in tris buffer (with sodium dioctyl sulfosuccinate) and centrifuged again at 2,520 g, for 15 min, at 20°C, in the same rotor. The sediments were again collected and suspended in buffered saline (0.9% NaCl, 0.01 M tris, pH 7.1, 0.00075% sodium dioctyl sulfosuccinate). During collection of the sediments, care was taken to discard the bottom fraction of the pellet, which consisted of chitinous particles and cytoplasmic polyhedra. The cytoplasmic polyhedral inclusion bodies have a greater specific gravity than nucleopolyhedra (Martignoni, 1967).

The clean polyhedron suspension was placed in a gallon bottle, in a refrigerator, and the polyhedra were allowed to sediment. At intervals of one or more days, the supernatant was decanted twelve times (by means of a Brewer automatic pipetting machine), with a total buffered saline replacement of 16 liters. The final suspension was ready for assay, formulation, and packaging.

Formulation, packaging, and storage

2.8 liters of the PIB suspension were diluted with an equal volume of 0.4% Cellosize QP 100 M, in buffered saline, with 0.00075% sodium dioctyl sulfosuccinate. The pH was adjusted to 7.0, the suspension was blended, and samples taken for enumeration of the polyhedra and other tests. As soon as the polyhedron count was determined, the suspension was transferred, by means of a Brewer automatic pipette and under constant stirring, to plastic (Nalgene) bottles. The volume dispensed (ml 419.5) was such that each bottle contained 15 acres doses (1.5×10^{12} PIBs). The bottles were capped, sealed with plastic tape, and stored at $-18^{\circ}C$.

Yield

The ratio of inclusion bodies produced to total amount of inoculum used for production purposes was about 10^3. In future production runs this ratio could be increased by one order of magnitude by decreasing the number of PIBs in the inoculum. The yield ratio based on the peroral LD_{90} was 4×10^5. The total PIB production (inclusion bodies

recovered after screening) was 26.1 X 10^{12}. The number of PIBs available after enrichment was 20.6 X 10^{12}. The loss during purification was 21%. Although quantitative data are not available, we estimate that a large part of this loss consisted of cytoplasmic polyhedra contained in the bottom fraction of the pellets after centrifugation: as mentioned above, this fraction was discarded during the enrichment process.

SAFETY EVALUATION

Toxicity-pathogenicity study

In planning for virus mass production and field application, and with the cooperation of the Agricultural Research Service, of the Fish and Wildlife Service, and of Hazleton Laboratories, we completed extensive toxicity-pathogenicity tests with the active viral ingredients. The methods used for our tests were standard procedures as recommended for testing water, milk, foods, drugs, and pesticides.

All of the following tests gave negative results:

(1) Acute oral toxicity tests (see, in part, Tucker and Crabtree, 1970)

- (a) mallards
- (b) pheasants
- (c) house sparrows
- (d) rats
- (e) albino rabbits
- (f) mule deer

(2) Acute eye application

- (a) albino rabbits (Draize test)

(3) Acute dermal application

(a) albino rabbits

(4) Acute intramuscular injection

(a) albino rabbits

(5) Acute intradermal injection

(a) guinea pigs

(6) Primary skin irritation

(a) albino rabbits

(7) Skin sensitization study

(a) guinea pigs (Landsteiner technique)

(8) Chronic feeding test

(a) honey bees (see Knox, 1970)

(9) Tissue culture studies

(a) bluegill (BF-2)
(b) bullhead (BB)
(c) minnow (FHM)
(d) bullfrog (FT)
(e) rainbow trout, embryonic (RTF-1)
(f) rainbow trout, subadult (RTG-2)

The tissue culture study (in cooperation with Dr. Kenneth E. Wolf, of the Eastern Fish Disease Laboratory) proved that six eurythermic cell lines are totally refractory to nucleopolyhedrosis virus. Not only did we find that the virus does not replicate nor it produces cytopathic effects in the culture cells (even after forced adsorption by the method of Weiss and Dressler), but we also proved that it is incapable of interference, when the cells were subsequently challenged

with infectious pancreatic necrosis virus, following exposure to nucleopolyhedrosis virus.

The results of our toxicity-pathogenicity testing program indicate that there is no reason to suspect that our preparation is unsafe for use as a pesticide in the forest environment. A summary of our tests was submitted to the virologists of the California Department of Public Health. The virologists reviewed our material and concurred that there should be no risk to human health from the use of the viral preparation as a microbial insecticide against the Douglas-fir tussock moth.

Production control

Before use, our viral preparation No. 49 was examined bacteriologically, to ensure that pathogenic agents (such as *Shigella* and *Salmonella*) were absent, and that the bacterial plate count of the preparation was within acceptable limits. The following tests were selected for this control procedure (Martignoni, 1970):

(1) Plate count on trypticase soy agar
(2) Spore count on trypticase soy agar
(3) Coliform count on Formula II Endo agar
(4) Fecal coliform test in EC medium
(5) *Shigella* and *Salmonella* test on SS agar
(6) Intraperitoneal injection in Swiss-Webster mice

The suspension (No. 49) had an aerobic bacterial count of 6.8×10^6 cells per 10^9 PIB. Cultures for fecal coliforms, *Shigella*, and *Salmonella* were negative. Intraperitoneal injections in mice were performed at two dose levels: one-acre equivalent (projected

dose) and 0.1-acre equivalent. Only one mouse in the one-acre equivalent group died on the day of inoculation. All other animals survived the 7-day observation period without discomfort.

INFECTIVITY TITER

The infectivity titer of the suspension was determined by biological assay in larvae of *Hemerocampa pseudotsugata* (our inbred strain "GL"). The larvae (75 ± 5 mg) were inoculated per os, as described previously (Martignoni, 1970). The median lethal dose, the LD_{90}, and the slope of the dose-mortality regression line were estimated according to Berkson's logit chi square method, by means of the Giese-Stewart LOCSAN computer program.

The median lethal dose of preparation No. 49 was 16 PIB/larva, or 208 PIB/gram larval weight. The computed slope b was 1.398, and the LD_{90} was estimated at 580 PIB/larva (see also table 1).

Several cases of cytoplasmic polyhedrosis occurred among the test insects, mostly as mixed infections, with nucleopolyhedrosis. Presence of a small amount of cytoplasmic polyhedrosis virus was also detected by electron microscopic examination of preparation No. 49. According to Mr. K. M. Hughes, who performed the electron microscope study, 93% of the polyhedral inclusion bodies were nucleopolyhedra (61.5% bundle virus strain, 31.5% single-rod virus strain), whereas 7% of the PIBs were cytoplasmic polyhedra.

As specified in the study plan, two sets of 10-ml samples of the diluted suspension were collected at the spray-system nozzle

immediately before and after application of each tankload, in the field. The samples collected after the application ("post-spray" samples) were to be used for bioassay. The samples collected before the application ("pre-spray" samples) were precautionary, in order to ensure that at least one set of samples would be available in case of accident or malfunction. Since the helicopter crashed before returning to the helispot, after the second application, only three sets of samples were available: two from the first tankload (pre-spray and post-spray samples) and one from the second tankload (pre-spray sample). The post-spray sample from the first tankload and the pre-spray sample from the second tankload were used for bioassay. Table 1 presents the results of all infectivity titrations, including the assay of the stock suspension before dilution with the spray formulation.

Table 1. Infectivity titers of three samples of preparation 49.

Sample	Bioassay No.	LD_{50} (PIB/larva)	95% fiducial limits (PIB) lower	upper
Stock suspension	338	15.6	1.2	44.6
Post-spray, first tankload	343	2.6	< 1	6.9
Pre-spray, second tankload	342	6.8	2.6	10.7

It is clear from Table 1 that there was no loss of activity in the field samples. Evidently, no inactivation occurred by mixing with the spray formulation and by exposure to the conditions prevailing in the helicopter spray equipment during application.

Polyhedron counts of the field samples were from 3 to 13% lower than the theoretically expected polyhedron counts. The loss could be caused, in part, by sedimentation and/or adhesion to spray-system components. Clumping did not appear to be a problem.

PLANNING AND COORDINATION

The several phases of virus production and production control were carefully planned, and adequate coordination between the steps was considered an important factor in achieving the intended goals within the prescribed time. In a somewhat simplified fashion, the work flow is schematically summarized in figure 1.

COOPERATORS

The following institutions and individuals donated much time and expertise for the successful outcome of certain phases of the work:

Dr. K. E. Wolf
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Eastern Fish Disease Laboratory
Leetown, West Va. 25430
(fish tissue culture)

Drs. D. Glen Crabtree & R. K. Tucker
U.S. Department of the Interior
Denver Wildlife Research Center
Denver, Colorado 80225
(tests for toxicity-pathogenicity to wildlife)

Mr. David Knox
U.S. Department of Agriculture
Entomology Research Division
Insect Pathology Laboratory
Beltsville, Maryland 20705
(tests for toxicity-pathogenicity to bees)

Other phases of the project were completed under contract by the following laboratories:

Hazleton Laboratories Falls Church, Virginia 22046	(tests for toxicity-pathogenicity to rats, guinea pigs, and rabbits)
U.S. Laboratories, Inc. Oakland, California 94601	(tests for acute toxicity-pathogenicity to mice)
Bio-Science Laboratories Van Nuys, California 91405	(tests for acute toxicity-pathogenicity to mice)

Among the Station personnel participating in this study, special mention should be made of Mr. Paul J. Iwai, for laboratory assistance, as well as Mrs. Dorothy E. Martin and Mr. Larry E. Stewart for computer analysis of the data.

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